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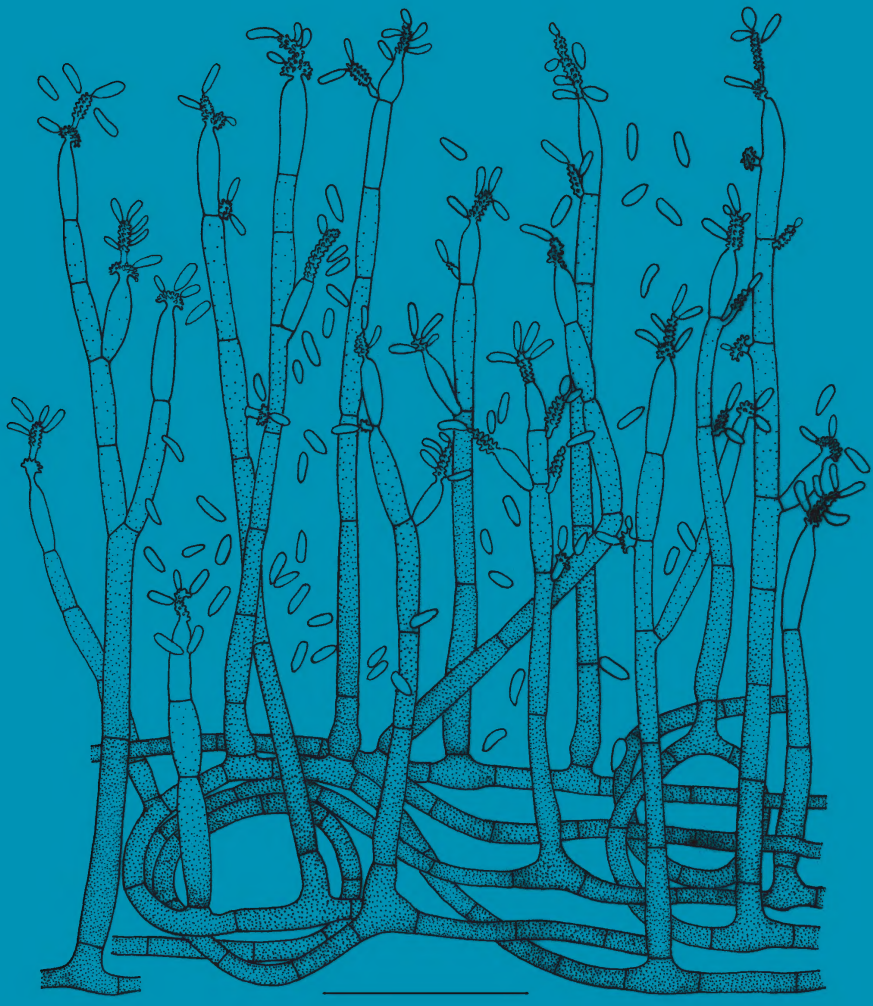

Rhinocladiella tibetensis sp. nov.
(Wu & al.— FIG. 2, p. 125)
YUE-MING WU, *artist*

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Reviewers — volume one hundred twenty-five

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this volume.

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MYCOTAXON *for* April–June, volume 124 (xii + 1–368)
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Errata from previous volumes

Volume 124

p. 46, line 20	FOR: 41°48'N 36°31'E	READ: 37°27'N 30°54'E
p. 69, last line	FOR: incubation period at 16–19°C ascomata developed. READ: incubation period at 16–19°C.	
p. 172, line 28	for: Fig. 1c	read: Fig. 2c
p. 172, line 31	for: Fig. 1d	read: Fig. 2d
p. 172, line 32	for: Fig. 1e	read: Fig. 2e
p. 178, line 3	for: Fig. 6a	read: Fig. 6b
p. 178, line 5	for: Fig. 6b	read: Fig. 6a
p. 179, line 39	FOR: 1993:	READ: 1993.
p. 192, line 20	FOR: Cácerese	READ: Cáceres
p. 207, line 37	FOR: Simbiosis	READ: Symbiosis
p. 210, line 15	FOR: (Gorjón & Hallenberg 2012) READ: (Gorjón & Hallenberg 2013)	

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From the Editor-in-Chief

Thoughts on authorities in taxonomic papers — Mycotaxon originated its usage and rules regarding authorities for taxonomic names at a time when there was no easy way of finding author citations. Our authors were expected to provide authorities for every taxon (fungal or not) at least once (usually at first mention). With the advent of the Internet and standardized abbreviations, however, mycologists now have easy access to Index Fungorum, MycoBank, and other sites that provide this information. I personally have been vexed to struggle through an introduction containing a sea of authorities for 'incidental' names and also crammed with text references, trying to sift the important information from the non-essential. It is now time to revise our citation requirements.

Shaun and I suggest that authorities for plant (and other non-fungal) names be either omitted entirely for incidental taxa or restricted (and cited once only) to the Specimens examined sections. When a significant host is to be cited frequently, then the proper authority should be given (once!) at first mention (usually in that overworked Introduction, unfortunately).

When it comes to fungi, however, our old rule applies for the nonce. We still require authorities be cited (once only) for all fungal names — either at first mention or within a table or appropriate taxonomic section.

Mycotaxon 125 contains 37 papers by 140 authors (from 20 countries) and revised by 54 expert reviewers. It also announces two new mycobiotas for its website.

Within these pages are two new genera with two new species (*Digitella* from Mexico, *Jayarambhatia* from India) plus 31 other species new to science representing *Drechslerella, Emericella, Geosmithia, Golovinomyces, Hydnotrya, Hygrocybe, Nephroma, Phyllosticta, Radulidium, Rhinocladiella, Scleroderma, Septobasidium, Setosynnema, Tuber,* and *Xylaria* from China; *Fuscoporia, Redbia,* and *Scutellinia* from Mexico; *Gilmaniella* from Malaysia; *Leptonia* from Australia; *Nidula* from India; *Repetophragma* from the Iberian peninsula; *Scutellospora* from Brazil; and *Sympodioplanus* from India.

In addition to range extensions for previously named taxa, we also offer new combinations in *Leptonia, Mattirolia, Micropeltis, Scolecopeltis,* and *Thyridium* as well as a validation of a *Gyroporus* from North America, a monograph of *Graphis* in Vietnam, and an excellent overview of how to provide the essential georeference data for all fungal collections.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
12 November 2013

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Re-appraisal of *Scolecopeltidium*

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Abstract — The five new species included in the original circumscription of *Scolecopeltidium* are re-examined, redescribed, and illustrated. *Scolecopeltidium mirabile* and *S. hormosporum* are retained in *Scolecopeltidium*, while *S. liciniae* is lectotypified and transferred to *Scolecopeltis*, and *S. costi* and *S. multiseptatum* are transferred to *Micropeltis*.

Key words — *Dothideomycetes*, morphological characters, taxonomy

Introduction

Scolecopeltidium, which was established by Stevens & Manter (1925), comprises species that lack superficial mycelium and are foliar epiphytes on leaves. In *Scolecopeltidium* thyriothecia are relatively large, circular, and blue-green with a central rounded irregular ostiole and poorly developed basal peridium. The upper thyrothecial wall consists of an irregular meandering arrangement of compact hyphae (Wu et al. 2011a). The dense filamentous hamathecium comprises pseudoparaphyses embedded in mucilage surrounding the asci, which are inclined towards the center. Asci are bitunicate or fissitunicate, obclavate, and pedicellate, lacking an ocular chamber, and containing fasciculate long cylindrical to filiform ascospores (Wu et al. 2011a). Stevens & Manter (1925) introduced eleven species in *Scolecopeltidium* (five new to science and six new combinations) but did not indicate any type for the genus. The earliest designation of a generic type was by Clements & Shear (1931), who selected *S. salacense* (Racib.) F. Stevens & Manter (and also proposed the superfluous replacement name, *Scolecopeltium*). *Scolecopeltidium* has until recently been listed as a genus of *Microthyriaceae*, although Lumbsch & Huhndorf (2010) suggested that the placement in this family is uncertain.

Batista (1959), who redescribed the genus, added many taxa and brought the total to 69 species. He retained *S. salacense* within *Scolecopeltidium* but designated *S. mirabile* as a substitute generic type; however this re-typification is inadmissible (ICN Art. 10.5; McNeill et al. 2012). Species were distinguished based on ascoma color and size and ascospore septation. Batista's (1959) placement of *Scolecopeltidium* in *Micropeltidaceae* was confirmed by Wu et al. (2011a). There are currently no molecular data for any *Scolecopeltidium* species.

Mistakenly following Batista (1959) in treating *S. mirabile* as the generic type, Wu et al. (2011a) provided a description and plate based on a 1931 Stevens collection. The correct generic type, *S. salacense*, is described as having 8-spored asci, copious paraphyses, and 6–12-septate ascospores (Raciborski 1900; Stevens & Manter 1925), but we have been unable to locate any authentic specimen of *S. salacense*.

We redescribe and illustrate the generic types of *Dothideomycetes* (Zhang et al. 2008, 2009, 2012; Wu et al. 2010, 2011a,b) and, where possible, have selected fresh specimens for epitypification and molecular analysis to provide a natural classification (e.g., Wu et al. 2011a; Chomnunti et al. 2011). In this paper, we re-examine the five new species described in *Scolecopeltidium* by Stevens & Manter (1925).

Materials & methods

Type specimens of the species were obtained from NY and ILL (for full names of herbaria see http://sweetgum.nybg.org/ih/index.php). The methods follow those of Wu et al. (2011a).

Taxonomy

Micropeltidaceae

Wu et al. (2011a) illustrated *Micropeltis applanata* Mont. (type species of *Micropeltidaceae*) and transferred *Byssopeltis* and *Scolecopeltidium* to the family. *Scolecopeltidium*, *Scolecopeltis*, and *Micropeltis* can be differentiated morphologically based on the presence or absence of pseudoparaphyses and shape and septation of ascospores. Studies at the molecular level are needed to confirm the separation of genera based on these characters.

Key to the genera of the *Micropeltidaceae* discussed in this paper

1. Ascospores either spindle-shaped to fusiform or filiform to long clavate; pseudoparaphyses (usually) present. 2
1. Ascospores spindle-shaped to fusiform; pseudoparaphyses absent . . *Scolecopeltis*
2. Ascospores >5-septate . . . . . *Scolecopeltidium*
2. Ascospores 2–5-septate . . . . . *Micropeltis*

Scolecopeltidium F. Stevens & Manter, Bot. Gaz. 79(3): 282 (1925)

TYPE: *Scolecopeltidium salacense* (Racib.) F. Stevens & Manter, Bot. Gaz. 79(3): 282 (1925) as "*salacensis*". [Designated by Clements & Shear 1931: 304, sub "*Scolecopeltium*".] [Type specimen not seen.]

FOLIAR EPIPHYTES on leaves, lacking superficial mycelium. THYRIOTHECIA relatively large, circular, scattered, membranaceous or submembraneous, superficial, blue-green to dark-green or black, base poorly developed, with a central rounded irregular ostiole; in section lenticular. UPPER WALL comprising an irregular meandering arrangement of compact hyphae. PERIDIUM a single stratum, comprising one to several layers of compact brownish black pseudoparenchymatous cells. HAMATHECIUM of dense, filamentous, pseudoparaphyses, embedded in mucilage, surrounding the asci, which are inclined towards the center. ASCI 2–8-spored, bitunicate, fissitunicate, obclavate, pedicellate, ocular chamber not observed. ASCOSPORES fasciculate, spindle-shaped or fusiform to long cylindrical or filiform, tapering towards the base.

ANAMORPHS: Not established for the genus (Hyde et al. 2011).

NOTES: We retain *S. mirabile* and *S. hormosporum* in *Scolecopeltidium*.

Scolecopeltidium mirabile F. Stevens & Manter, Bot. Gaz. 79(3): 283 (1925)

This species was described and illustrated in Wu et al. (2011a) based on a collection of Stevens, on leaves of *Diospyros aherni* from Philippines on 18 January 1931, NY 2021. Although the host and distribution differ, there are only minor differences between the original description (specimens from Kartabo, Guyana) and the 1931 Stevens specimen described in Wu et al. (2011a). Although Wu et al. described the 1931 Stevens specimen's asci as 8-spored and the ascospores as having 28–38 septa, the original description cited asci as 2–4-spored and ascospores as having up to 48 septa.

Scolecopeltidium hormosporum F. Stevens & Manter, Bot. Gaz. 79(3): 283 (1925) PLATE 1

FOLIAR EPIPHYTES on the upper surface of leaves, lacking superficial mycelium. THYRIOTHECIA 1940–2960 µm diam × 75–95 µm high, relatively large, scattered, superficial, carbonaceous, rounded from above, blue-green to black, base wall poorly developed, with a central rounded irregular ostiole; in section lenticular. UPPER WALL comprising an irregular meandering arrangement of compact hyphae. PERIDIUM 16.8–22.5 µm wide, a single stratum, composed one to several layers of brown-black and compact pseudoparenchymatous cells. HAMATHECIUM of dense, 1–2 µm wide, filamentous, branched pseudoparaphyses embedded in mucilage, longer than, and surrounding the asci. ASCI 90–125 × 13.5–33 µm when young, 175–195 × 33.5 × 54.5 µm (mean = 159.9 × 43.9 µm, n = 15) at maturity, 3–6-spored, fusiform, pedicel short, with a distinctive but small ocular chamber. ASCOSPORES 150–160 × 8.5–9.5 µm (mean = 156.5 × 9 µm, n = 20),

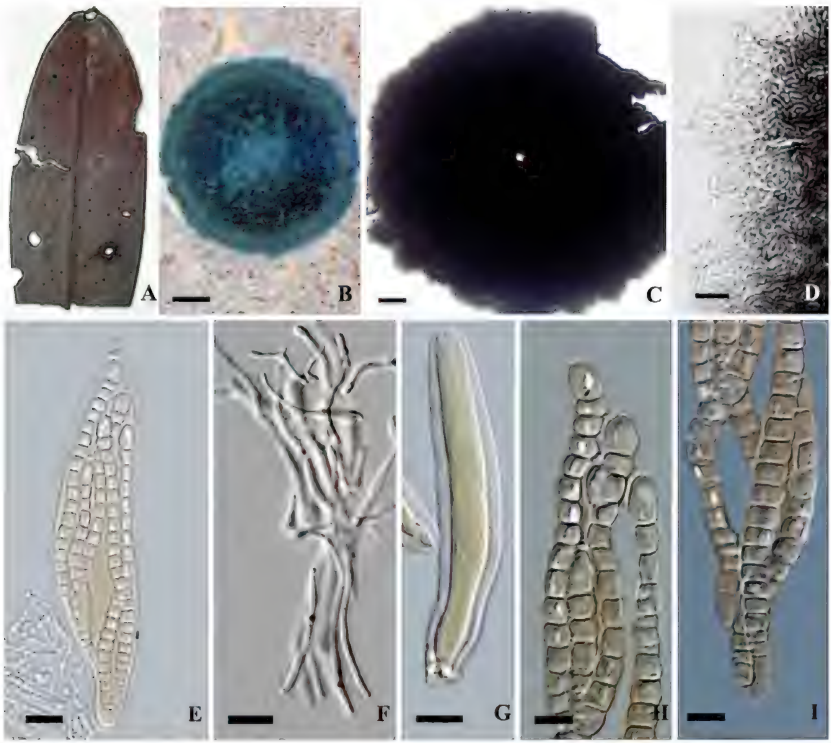

PLATE 1. *Scolecopeltidium hormosporum* (holotype, ILL6779). A, B. Appearance of ascomata on the host leaf surface. C, D. Squash mounts of ascoma showing strutures of tissues. E, G. Asci. F. Pseudoparaphyses. H, I. Ascospores with numerous septa. Scale bars: B = 500 μm, C, E = 20 μm, D, F–I = 10 μm.

filiform to long cylindrical, apex widest (about 9.5 μm), end cells slightly elongate and wide, other cells spherical, hyaline, with 22–28 septa, constricted at septa, and curved in the ascus, immature spores with only one cell.

MATERIAL EXAMINED: **GUYANA** (British Guiana), Kartalo, on unknown host, 23 July 1922, F.L. Stevens No. 581 (ILL6779, holotype).

Scolecopeltis Speg., Boln. Acad. nac. Cienc. Córdoba 11(4): 574 (1889)

TYPE SPECIES: *Scolecopeltis tropicalis* Speg., Boln. Acad. nac. Cienc. Córdoba 11(4): 574 [no. 369] (1889)

FOLIAR EPIPHYTES on leaves, lacking superficial mycelium. THYRIOTHECIA, circular, scattered, membranaceous or submembraneous, superficial, blue-green to black brown, base poorly developed, with a central rounded ostiole; in section lenticular. UPPER WALL composed of irregularly meandering and interwoven compact hyphae. Peridium a single stratum, composed of one to several layers of compact brownish black pseudoparenchymatous cells. PSEUDOPARAPHYSES

absent. Asci (4–) 8-spored, bitunicate, fissitunicate, obclavate, pedicellate, ocular chamber not observed. Ascospores, overlapping, spindle-shaped to fusiform, with more than 10 septa.

Anamorphs: None reported.

Notes: *Scolecopeltis* differs from *Scolecopeltidium* in lacking a hamathecium of pseudoparaphyses (Stevens & Manter 1925). We transfer *Scolecopeltidium liciniae* to *Scolecopeltis*.

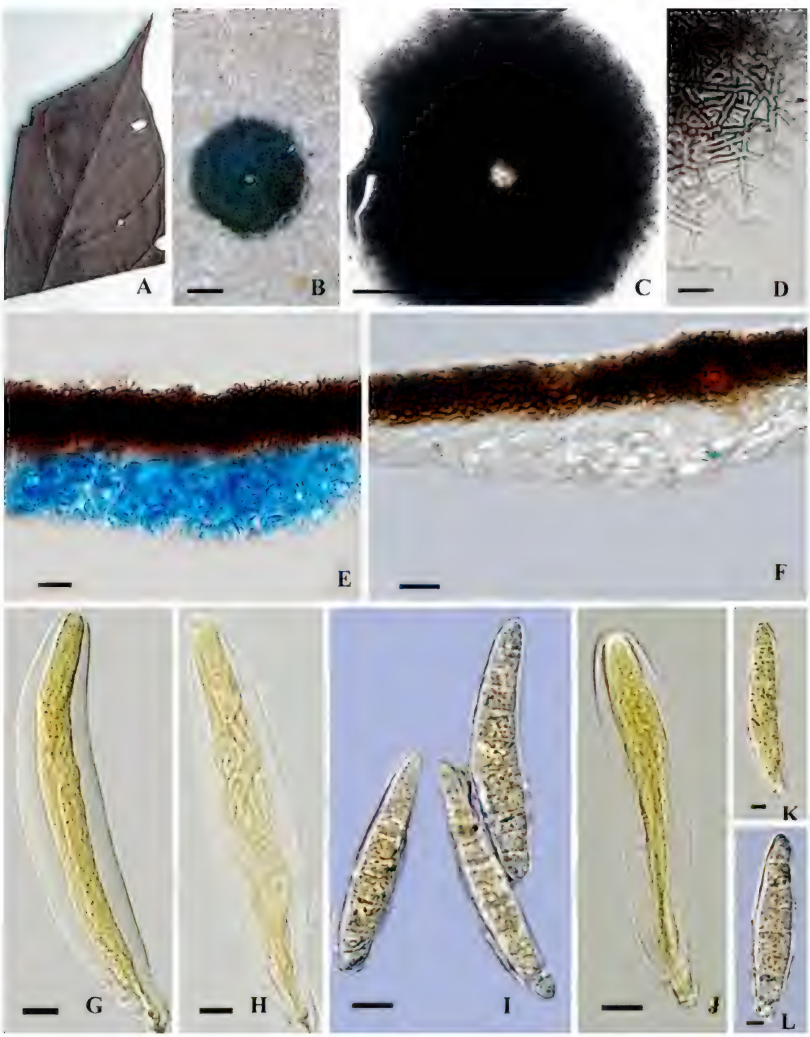

Plate 2. *Scolecopeltis liciniae* (lectotype, ILL6780). A, B. Appearance of ascomata on the host surface. C, D. Squash mount of ascomata. E, F. Section of ascomata. G, H, J. Immature asci. I, K, L. Ascospores. Scale bars: B = 500 µm, C = 200 µm, D–J = 10 µm, E = 20 µm, K, L = 5 µm.

Scolecopeltis liciniae (F. Stevens & Manter) H.X. Wu & K.D. Hyde, **comb. nov.**

MycoBank number: MB801935 Plate 2

≡ *Scolecopeltidium liciniae* F. Stevens & Manter, Bot. Gaz. 79(3): 283 (1925)

Type: Guyana (British Guiana), Rockstone, on leaves of *Licinia* sp., 17 July 1922, F.L. Stevens No. 479 (**Lectotype designated here**, ILL6780).

Foliar epiphytes on the lower surface of leaves of *Licinia* sp. (*Liliaceae*), lacking superficial mycelium. Thyriothecia 1500–1600 μm diameter, scattered, superficial, blue-green, membranaceous or submembraneous, rounded, within loosely interwoven hyphae at the margin, dome-like in section, with a rounded ostiole. Peridium 14.5–16.5 μm thick, composed of interwoven hyphae, lacking at the base. Hamathecium of sparse, paraphyses absent, surrounded by mucilage and themselves surrounding asci, which form from the tissues at the circumference and base. Asci 99–139 × 20–27.5 μm (mean = 115.5 × 23.3 μm, n = 15), 8-spored, cylindro–clavate, with a short pedicel about 1.5 × 1 μm, ocular chamber not observed. Ascospores 65.7–76.8 × 8.1–12 μm (mean = 69.9 × 10.1 μm, n = 20), overlapping, spindle-shaped to fusiform, 11–13 septa, rounded towards the apex, tapering towards the base, middle cells wider, conical, hyaline, slightly constricted at septa.

Notes: Stevens & Manter (1925) cited two collections [syntypes] in the protologue of *S. liciniae*: No. 423 [ILL 6781] and No. 479 [ILL 6780], both conserved in Stevens' herbarium at ILL. However, ILL 6781 represents a different species with pseudoparaphyses and 3-celled ascospores. We therefore designate ILL 6780 (on *Licinia* sp.) as lectotype.

Micropeltis Mont., Ann. Sci. Nat., Bot., sér. 2, 17: 122 (1842)

Type species: *Micropeltis applanata* Mont., Annls Sci. Nat., Bot., sér. 2, 17: 122 (1842)

Foliar epiphytes on the surface of leaves, superficial mycelium absent or not observed. Thyriothecia circular, gregarious, superficial, membranous, bluish or greenish to black, lower peridium poorly developed, easily removed from the host surface, with a central irregular ostiole; lenticular in section. Upper wall composed of irregularly meandering compact hyphae. Peridium black to light brown, comprising two strata, outer cells black and compact, inner cells comprising a textura angularis of loose light brown cells. Hamathecium consisting of asci embedded in mucilage and inclined toward the central ostiole with sparse or evanescent pseudoparaphyses present. Asci 6–8-spored, bitunicate, fissitunicate, obclavate to widely fusiform, pedicellate, ocular chamber not obvious. Ascospores overlapping 2–3-seriate, hyaline, long clavate to elliptic, trans-septate, smooth-walled.

Anamorphs: None reported.

Notes: *Micropeltis* differs from *Scolecopeltidium* in having fewer septa in its ascospores (Stevens & Manter 1925). We transfer *Scolecopeltidium costi* and *S. multiseptatum* to *Micropeltis*.

Micropeltis costi (F. Stevens & Manter) H.X. Wu & K.D. Hyde, **comb. nov.** Plate 3

MycoBank number: MB801936

≡ *Scolecopeltidium costi* F. Stevens & Manter, Bot. Gaz. 79(3): 284 (1925)

Foliar epiphytes on the upper surface of leaves, lacking superficial mycelium. Thyriothecia 830–1120 µm diam × 45–51 µm high, rounded,

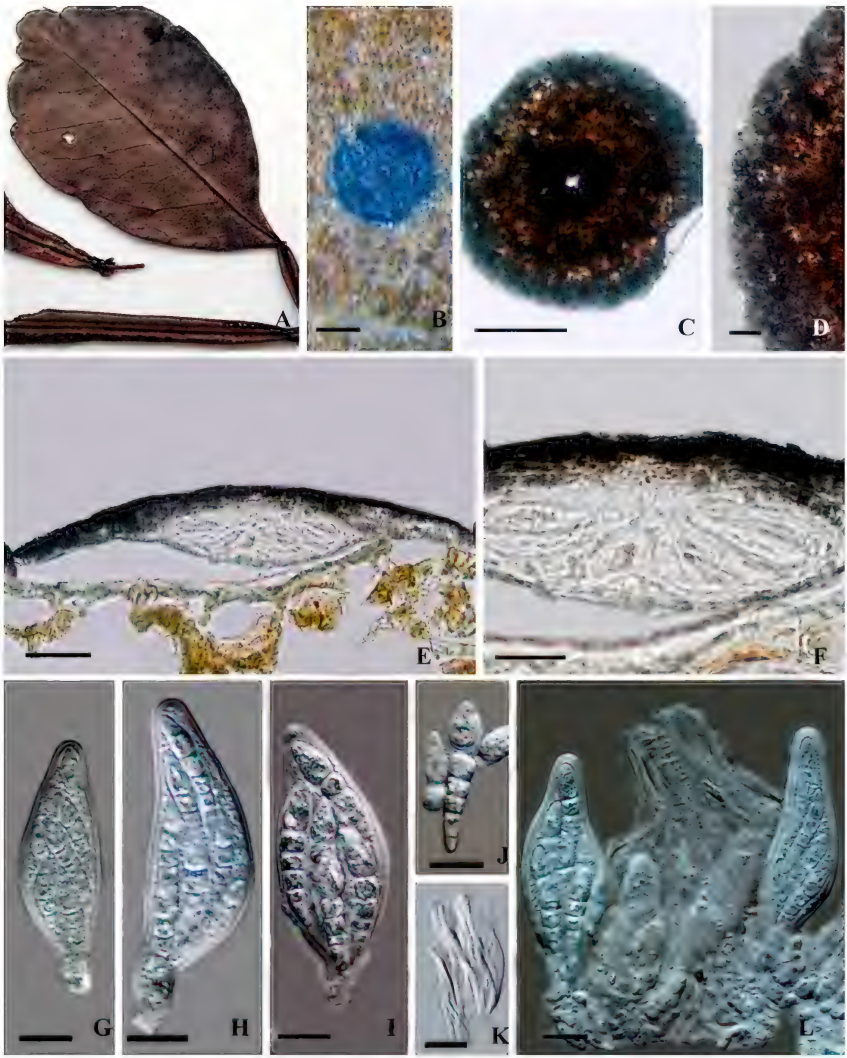

Plate 3. *Micropeltis costi* (holotype, NY585). A, B. Appearance of colony and thyriothecia on the host surface.C, D. Squash mount of ascoma. E, F. Section of ascomata. G–I, L. Asci. J. Ascospores with 4–5 septa.K. Pseudoparaphyses. Scale bars: B = 500 µm, C = 60 µm, D,F = 20 µm, E = 40 µm, G–L = 10 µm.

scattered, membranaceous or submembraneous, superficial, blue-green, basal peridium lacking, with a central rounded ostiole; dome-like in section. Upper wall comprising an irregular meandering arrangement of hyphae, with loosely interwoven hyphae at the margin. Peridium 5–10 μm wide, a single stratum, composed one to several brownish black to light brown layers outside compact pseudoparenchymatous cells. Hamathecium of dense, filiform 2 μm wide pseudoparaphyses, embedded in mucilage, surrounding the asci inclined towards the center. Asci 40–58 ×11–22μm (mean = 50.7 ×15.8 μm, n = 15), 8-spored, bitunicate, fissitunicate dehiscence not observed, broadly fusiform, with a 5 μm wide × 6 μm long pedicel. Ascospores 20–28 × 4–5 μm (mean = 22.8 × 4.5 μm, n = 20), 4–5 overlapping, elliptic or spindle-shaped, hyaline, 4–5 septa, slightly constricted at septa, with middle cells larger and round, smooth-walled.

Material examined: **GUYANA** (British Guiana), Kartabo, on leaves of *Serjania paucidentata*, 23 July 1922, F.L. Stevens (NY585, holotype).

Micropeltis multiseptata (F. Stevens & Manter) H.X. Wu & K.D. Hyde, **comb. nov.**

MycoBank number: MB801937 Plate 4

≡ *Scolecopeltidium multiseptatum* F. Stevens & Manter, Bot. Gaz. 79(3): 283 (1925)

Foliar epiphytes on the upper surface of leaves, lacking superficial mycelium. Thyriothecia 1270–1350 μm diameter × 76–80.5 μm high, scattered, superficial, blue-green, membranaceous or submembranaceous, basal peridium lacking, with a central rounded ostiole; dome–like in section. Upper wall comprising an irregular meandering arrangement of hyphae, with loosely interwoven hyphae at the margin. Peridium 19.5–21.5 μm thick, composed three layers of compact pseudoparenchymatous cells in which the outer layer is black, the middle of layer is blue-green, and the inner layer is hyaline. Hamathecium of dense filamentous crooked pseudoparaphyses, 1–1.5 μm wide, embedded in mucilage, surrounding the asci. Asci 69–80 × 22–25 μm (mean = 73 × 23.7 μm; n = 15), 2–4-spored, obclavate to widely fusiform, with a 3–4 μm wide × 4–5 μm long pedicel. Ascospores 27–37 × 8–9.5 μm (mean = 35.5 × 8.5μm, n = 20), 2–3-seriate, elliptic or spindle-shaped, slightly constricted at the septa, hyaline, with 3–4 septa.

Material examined: **GUYANA** (British Guiana), Wismar, on leaves of *Philodendron* sp., 24 July 1922, F.L. Stevens (NY 1004, isotype).

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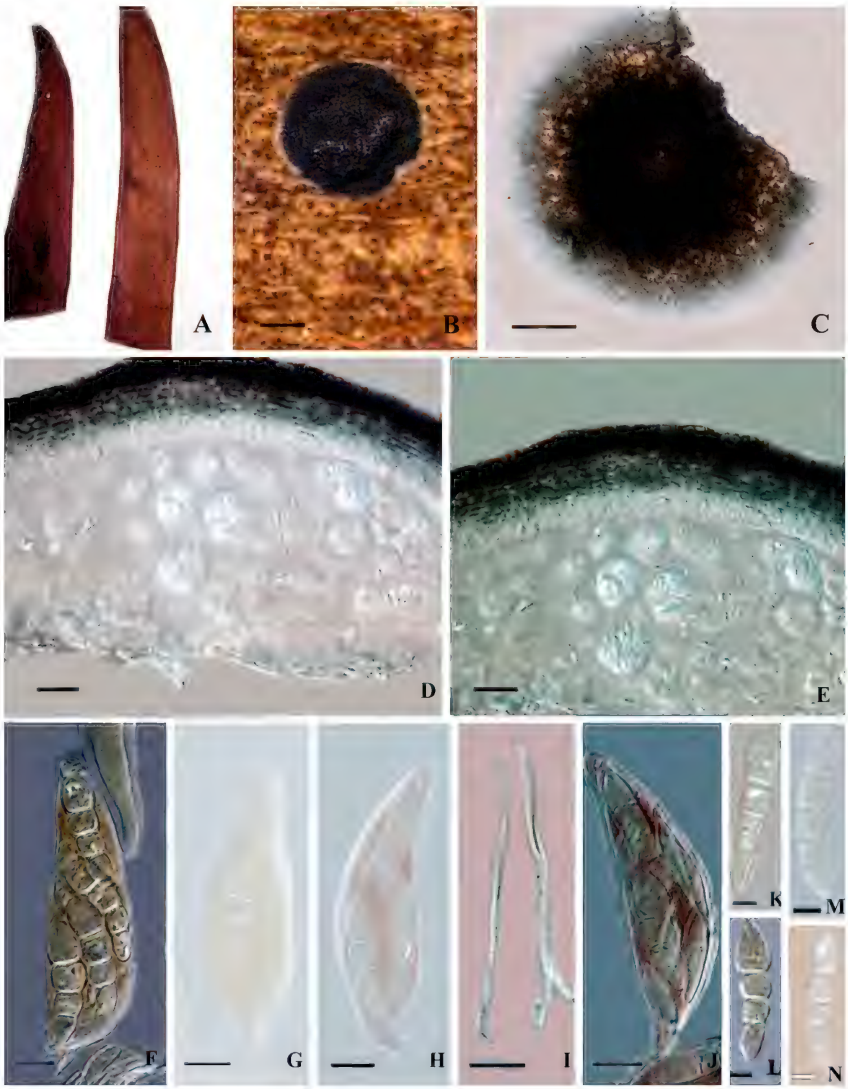

Plate 4. *Micropeltis multiseptata* (isotype, NY 1004). A, B. Appearance of ascomata on the host surface. C. Squash mount of ascoma. D, E. Section of ascoma. The peridium comprises three layers. F–-H, J. Obclavate asci. I. Pseudoparaphyses. K–N. Ascospores with 4–5 septa. Scale bars: B = 500 µm, C = 30 µm, D–J = 10 µm, K–N = 5 µm.

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Five *Leptonia* species from New South Wales and Queensland, Australia

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Abstract — Descriptions and illustrations are provided for five *Leptonia* species. Three species are new to science: *Leptonia boardinghousensis* possesses unusual basidiomes colours, *L. ambigua* has subisodiametric basidiospores that atypically have obscure angles at the apex and a lignicolous habit, and *L. omphalinoides* is distinguished by its lignicolous omphalinoid tiny basidiomes. Two species were previously reported: *L. poliopus* from Europe and *L. umbraphila* comb. nov from the Seychelles.

Key words — *Basidiomycota*, *Entolomataceae*, mtSSU, LSU, RPB2

Introduction

Surveys of species in the *Entolomataceae* Kotl. & Pouzar were conducted during April–May of 2010–12 in the temperate rainforests of central New South Wales and February–March 2009–12 from various localities within the Wet Tropics Bioregion throughout northeastern Queensland. Among the 918 entolomatoid collections obtained during the surveys, species of *Leptonia* (Fr.) P. Kumm. are numerous. Here we describe three new species, *L. boardinghousensis*, *L. ambigua*, and *L. omphalinoides* and report *L. poliopus* and *L. umbraphila* new for Australia.

The generic concept of *Leptonia* remains uncertain, because phylogenetic analyses do not support monophyly for species included in the genus and the major sub-clades containing *Leptonia* spp. lack statistical support (Co-David et al. 2009, Baroni & Matheny 2011). Until resolution of the generic

Table 1. Collection identifier and GenBank accession numbers of *Leptonia* species.

Species	Collection	GenBank accession numbers		
		mtSSU	LSU	RPB2
L. boardinghousensis	Largent 9875	—	JQ756410	JQ756425
	Largent 9926	JQ756397	JQ756411	JQ756426
	Largent 9935	—	JQ756412	JQ756427
	Largent 10086	JQ756398	JQ756413	JQ756428
L. ambigua	Largent 9872	JQ756399	JQ756414	JQ756429
	Largent 10130	—	JQ756415	JQ756430
L. omphalinoides	Largent 9673	JQ756400	JQ756416	JQ756431
	Largent 9800	JQ756401	JQ756417	JQ756432
L. poliopus	Largent 10209A	JQ756402	JQ756418	JQ756433
	Largent 10209B	JQ756403	JQ756419	JQ756434
	Largent 10209C	JQ756404	JQ756420	JQ756435
L. umbraphila	Largent 9629	JQ756405	—	JQ756436
	Largent 9630	JQ756406	JQ756421	JQ756437
	Largent 9640	JQ756407	JQ756422	JQ756438
	Largent 9766	JQ756408	JQ756423	JQ756439
	Largent 9793	JQ756409	JQ756424	JQ756440

and phylogenetic concepts, we follow Largent (1994), who recognizes the following combination of morphological characters for *Leptonia:* a habit that is typically mycenoid, collybioid, omphalinoid, or (rarely) tricholomatoid; a pileus that is squamulose or tomentulose to tomentose at least on the disc; a convex, parabolic, broadly convex, or convex-depressed pileus shape; pileipellis typically composed of erect hyphae at least on the discs of young basidiomes; inflated pileocystidia averaging >10 µm in diameter; lipoid globules and oleiferous hyphae absent or (if present) not abundant; aborted basidia typically absent.

Materials & methods

Macromorphological and micromorphological features

Colors of basidiomes were described subjectively and coded according to Kornerup & Wanscher (1978: 196–225, Diagrams Section), with color plates noted in parentheses. In descriptions color plates are abbreviated to indicate the page number, column(s), and row(s) [e.g., 8D–F5-6 = page 8, columns D–F, rows 5–6.] Common color names are from the charts at the top of each page, with technical color names enclosed by quotation marks at the bottom of each page in the Color Names subsection [e.g., the common name for plate 1, column A, row 2 is yellowish white, and the technical name is 'milk white'].

Measurements include: the arithmetic means ($\bar{x}$) of spore lengths and widths ± one standard deviation in n objects measured; the quotient of spore length by width (E) indicated as a range variation in n objects measured and the mean of E-values (Q)

± standard deviations. The sample size (N) equals the total number of microscopic structures measured (x) and number of basidiomes studied (y) and follows the format N = x/y. The following Q values define general basidiospore shape: isodiametric—Q = 1.0–1.15; subisodiametric—Q = 1.16–1.27; heterodiametric—Q >1.27 (Largent 1994).

The techniques and equipment for collecting data in the field, measuring basidiospores, and obtaining digital photographs of microscopic features follow Largent et al. (2011a).

All collections from New South Wales cited in the 'Additional collections examined' are deposited in The Plant Pathology Herbarium, Orange Agricultural Institute (DAR); Queensland collections were split, with duplicates deposited in the Australian Tropical Herbarium (CNS) and The Queensland Herbarium (BRI). All holotype and isotype collections are deposited in the herbaria designated using acronyms used in Thiers (2012).

DNA sequences

DNA extraction and Polymerase Chain Amplification (PCR) protocols for the mitochondrial small subunit of the ribosomal DNA (mtSSU), variable domains (D1, D2) of the nuclear large subunit (LSU), and the second largest subunit of the RNA polymerase gene (RPB2) follow Largent et al. (2011, 2013). For RPB2 amplification we used primers rpb2-i6f and rpb2-i7r (Co-David et al. 2009). Alternatively, we used additional forward RPB2 primers (rpb2-EntF1 and rpb2-EntF2; Largent et al. 2013) in combination with one of three reverse primers: rpb2-EntR1b (5′ to 3′– ATG GAT YTC RCA RTG TGT CCA), rpb2-EntR2 (5′ to 3′– GTG GAT TTC RCA RTG AGT CCA), or rpb2-EntR4 (5′ to 3′ – TGA ATY TCR CAR TGC GTC CCA). Sequences were generated on an Applied Biosystems 3130xl Genetic Analyzer at Middle Tennessee State University using the sequencing and precipitation protocols outlined in Largent et al. (2011a,b). Accessions for sequences obtained from the three genes are provided in Table 1.

Taxonomy

Leptonia boardinghousensis Largent, **sp. nov.** Plates 1–2

MycoBank MB 802806

Differs from *Inocephalus quadratus* by its basidiome colors, long rostrate-ventricose opaque hymenial cystidia, and heterodiametric 6–7(–8)-angled basidiospores.

Type — Australia, New South Wales, Watagans National Park, Boardinghouse Dam Track within 20 m of 32°59′57″S 151°24′15″E, 12 April 2011, DL Largent 10086 (**holotype**, DAR).

Etymology — referring to Boardinghouse Dam Track in Watagans National Park.

Pileus 8–40 mm broad, 3–14 mm high, dull, always opaque (not translucent) and not hygrophanous; in young basidiomes convex to broadly convex then broadly parabolic, irregularly campanulate, plano-convex, and eventually uplifted, rarely obscurely umbonate in expanding and maturing basidiomes; at first entirely tomentulose or tomentulose-squamulose, in expanding basidioma remaining tomentulose to tomentulose-squamulose on or near the disc, squamulose towards the margin and appressed-fibrillose-striate at the margin,

eventually in mature forms becoming appressed-fibrillose and then striate and rimose from the margin to near the disc; entirely orange (6A6) in young and protected basidiomes, in exposed basidiomes the tomentum, squamules, and fibrils at first dark brown with a reddish tone (7 or 8F6–7), becoming a bit lighter (7 or 8E5–7) and eventually fading to light brown (6–7C-D3–6); margin incurved to more typically decurved then uplifted, eventually wavy to lobed and split in mature forms; context reddish white to pale red (6–7A-B2–5) becoming more reddish in a rimose surface, 0.5–1.0 mm thick above the stipe, nearly non-existent at the margin. Odor weakly fungal, at times faintly pleasant or fragrant. Taste slightly unpleasant or bitter and astringent. Lamellae 3–20 mm long, 1–7 mm deep, adnexed, close to subdistant and narrow to moderately broad at first, then subdistant and sigmoid, ventricose, or broad with maturity, at first pinkish white (6–7A2) darkening to brownish orange (5–7A3) and then pale red to pastel red (7A, B or C3–4) with basidiospore maturity; margin typically smooth and concolorous, in some lighter colored and faintly fimbriate. Stipe 25–72 mm long, 0.75–5.0 mm broad at apex, middle, and base, in some flattened and longitudinally grooved and then at the apex 5–6 × 2 mm at the apex, 4 × 2 or 5 × 4 mm broad in the middle, and 3 × 2.5 or 5 × 4 mm broad at base, equal to often tapered with the apex smaller or in one with the base smaller, pruinose at the apex, smooth to the eye but weakly longitudinally striate under 10×, at first pale orange to pale red (5–7A3–4), remaining so at the apex, but darkening (5–7A-B3–5) with maturity; hollow, typically sturdy and stiff, rarely fragile and splitting longitudinally; basal tomentum white, typically at first scarce, often remaining so with age but at times becoming moderate to abundant in basidiomes in deep humus, rarely absent and then only in exposed basidiomes found in shallow or compacted humus along trails. Bruising Reactions stipe browning when handled.

Basidiospores in profile view 6–7(–8)-angled, angles somewhat knobby, subheterodiametric to heterodiametric, in end view 6-angled and isodiametric, 8.1–11.7 × 6.0–8.9 µm (x = 9.7 ± 0.7 × 7.2 ± 0.53 µm; E = 1.13–1.62; Q = 1.36 ± 0.10 (heterodiametric); n = 112/4). Basidia clavate, 4-sterigmate, in some with fairly large granules, 32.1–43.5 × 6.4–11.2 µm (x = 38.6 ± 3.9 × 9.6 ± 1.6 µm; E = 3.5–5.2; Q = 4.1 ± 0.60; n = 13/2). Cheilocystidia versiform, clavate, obclavate, clavate and acuminate, ventricose-rostrate, or ventricose-rostrate with the rostrum strangulated and tapered, thin-walled to slightly thick-walled, colorless, scattered to abundant, 51.3–111.4 × 10.1–32.1 µm (E = 3.16–6.30; Q = 4.2 ± 0.77; n = 15/2). Pleurocystidia as pseudocystidia, similar in shape but typically longer than the cheilocystidia, often originating from the outer portions of the lamellar trama, not associated with the oleiferous hyphae, opaque but colorless, 81.3–147.6 × 12.5–18.5 µm (E = 3.6–7.6; Q = 5.5 ± 1.4; n = 11/2). Lamellar tramal hyphae subparallel, rather long and broad,

Plate 1. *Leptonia boardinghousensis* {DLL 10086 holotype}. A: Basidiomata habit; B: Lamellar attachment (note whitish edges on maturing lamellae and pruinose stipe apex); C: Pileus surface, mature surface tomentulose to tomentulose-squamulose on the disc, appressed fibrillose elsewhere (left), immature surface, entirely tomentulose (right). Bar (A-C) = 10 mm.

without lipoid globules, 49.1–305.2 × 3.7–32.2 µm (N = 14/2). PILEIPELLIS a layer of entangled hyphae, in young basidiomes more or less erect, in older basidiomes erect to suberect in the center of pileus and semi-erect towards the margin, and repent at the margin. PILEOCYSTIDIA cylindro-clavate to clavate to broadly clavate, 27.8–180.6 × 6.0–19.8 µm (E = 2.0–13.5; Q = 6.6 ± 3.8; N = 18/3). PILEAL TRAMAL HYPHAE entangled, similar in size and shape to the lamellar trama. STIPITIPELLIS with rare to scattered clusters of hymenial elements at the apex, a cutis elsewhere. CAULOCYSTIDIA absent. OLEIFEROUS HYPHAE rare, scattered, or absent in the pileal and lamellar trama. LIPOID GLOBULES absent. BRILLIANT GRANULES absent. PIGMENTATION in the pileipellis cytoplasmic as well as faintly externally incrusted on the outer hyphal walls. CLAMP CONNECTIONS absent in all tissues.

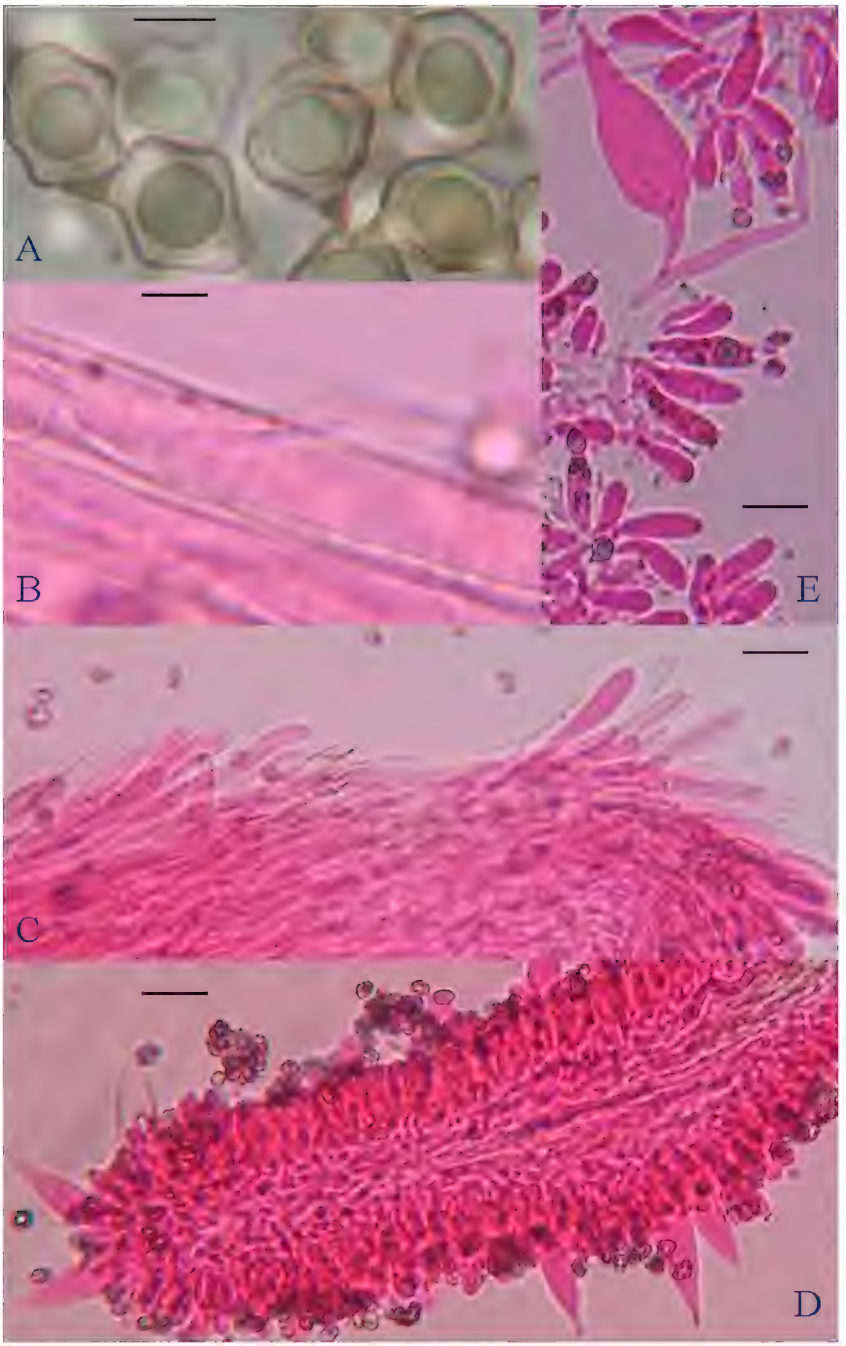
ECOLOGY & DISTRIBUTION — Rarely solitary, more often scattered, gregarious, or even caespitose in soil amongst leaf litter, in wet sclerophyll, warm temperate gallery rainforest; central or northern Hunter District, New South Wales, April–May.

ADDITIONAL COLLECTIONS EXAMINED — **AUSTRALIA. NEW SOUTH WALES**, central Hunter District, Barrington Tops National Park, Williams River Day Use Area, end of Blue Gum Track, 32°09′03.4″S 151°31′35.1″E, 14 April 2010, DL Largent 9875; 32°09′08′.3″S 151°31′37.0″E, DL Largent 9877; 32°09′03.2″S 151°31′23.6″E, 18 April 2010, DL Largent 9894; 32°09′03.2″S 151°31′23.6″E, 4 May 2011, DL Largent 10193. Williams River Day Use Area, Track to Pool of Reflections, 32°08′15.8″S 151°30′38.2″E, 22 April 2010, DL Largent 9935. Myall Lakes National Park, Seal Rock Road, 32°25′08″S 152°28′26″E, 6 May 2011, DL Largent 10203. Watagans National Park, Boardinghouse Dam Track, 32°59′57.7″S 151°24′15.9″E, 13 April 2010, DL Largent 9867; 32°59′57.8″S 151°24′15.4″E, 21 April 2010, DL Largent 9926; 32°59′57.7″S 151°24′15.8″E, 28 April 2010, DL Largent 9958; 32°59′58.2″S 151°24′15.4″E, DL Largent 9959; 32°59′59′.2″S 151°24′16.3″E, DL Largent 9960; 33°00′00.7″S 151°24′17.3″E, DL Largent 9961; 32°59′57″S 151°24′17″E, 23 April 2011, DL Largent 10144, 10145; 32°59′57″S 151°24′17″E, 1 May 2011, DL Largent 10175.

DISTINCTIVE CHARACTERS — The basidiome color changes of orange then pinkish to reddish in the stipe, orange then dark brown with a reddish tone in the pileus, pinkish white in the lamellae, and reddish white to pale red in the pileal context; an entirely tomentulose pileal surface at first that becomes striate to the margin with maturity; basidiospores 6–7-angled and averaging <10 µm × <7.5 µm; versiform hymenial cystidia; and clampless hyphae.

COMMENTS — In 2010 and 2011, several *L. boardinghousensis* populations of 10–20 basidiomes each were common throughout the Boardinghouse Dam

PLATE 2. *Leptonia boardinghousensis* {DLL 9925 A-B, D; DLL 9867 C,E}. A: Basidiospores; B: Faintly incrusted pigment on hyphae of pileipellis; C: Pileipellis and pileocystidia near pileal margin; D: Cheilocystidia, pleurocystidia, and oleiferous hyphae in lamellar trama; E: Ventricose-rostrate pleurocystidia with rostrum strangulated and tapered, basidia with sterigma, basidioles in squash mount. Bars: A = 5 µm; B = 25 µm; C = 25 µm; D = 40 µm; E = 25 µm.


A
B
E
C
D

Track in Watagans National Park. Collections found in relatively deep humus and protected by a dense overstory regularly consisted of large basidiomes with pilei up to 40 mm broad and stipes 35–75 mm long and with orange colors dominant. Collections in more open areas with less humus consistently produced smaller pilei (<28 mm broad) and shorter stipes (25–55 mm long) with brown to reddish brown color predominating.

The pantropical *Inocephalus quadratus* (Berk. & M.A. Curtis) T.J. Baroni resembles *L. boardinghousensis* macroscopically but differs microscopically in its cuboidal basidiospores and cylindro-clavate to clavate cheilocystidia (Baroni & Halling 2000, Noordeloos & Hausknecht 2007).

Leptonia ambigua Largent, **sp. nov.** PLATES 3–4

MYCOBANK MB 802807

Differs from *Leptonia tjallingiorum* by smaller basidiomes, the dark greyish violet to blackish blue pileus, the subisodiametric basidiospores, and the absence of cheilocystidia and clamp connections in the pileipellis.

TYPE — Australia, New South Wales, Barrington Tops National Park, end of Blue Gum Track, within 20 m of 32°09′03.8″S 151°31′30.2″E, 21 April 2011, DL Largent 10130 (**holotype**, DAR).

ETYMOLOGY — derived from the Latin *ambiguus* (= ambiguous), referring to the obscure angles at the apex of some basidiospores.

PILEUS 8–33 mm broad, 5–10 mm high; opaque and not translucent, not hygrophanous; typically plane, at times obscurely umbonate, never depressed; when young convex to parabolic, upon expansion and maturity becoming broadly convex and eventually plane; at first entirely densely matted fibrillose to matted tomentulose then remaining matted tomentulose in the center and becoming appressed squamulose elsewhere, and eventually appressed fibrillose at or near the margin, not striate; at first entirely dull violet to near dark violet to between dark greyish violet and blackish blue (18E4 or 19E–F3–4), remaining so on the disc but the squamules and fibrils fade to greyish lilac (16D2) or purplish grey (14–15C2), eventually the violaceous color fades completely and the pileus becomes brown (6–7D-F3–4); context pallid, 1 mm thick above the stipe, nearly non-existent at the margin; margin decurved then plane. ODOR indistinct. TASTE indistinct, mild. LAMELLAE 5–13 mm long, 1-5 mm deep, adnexed or narrowly adnate at all times, close then subdistant, narrow then moderately broad, at first violet white (18A2), quickly becoming reddish grey (7A2), then white to orange white (5–7A1–2), and eventually pale brownish orange (6C4) with basidiospore maturity; margin smooth and concolorous. STIPE 22–54 mm long, apex 1–3 mm broad, base 1–4 mm broad, equal to slightly enlarged towards the base, longitudinally appressed fibrillose except for the pruinose apex, at first the fibrils dull violet (18E4, 18D3–4) then violet grey (16D2 or 16B2) and eventually dull red (9B3) or reddish grey (9C2);

Plate 3. *Leptonia ambigua* {DLL 10130 holotype}. A: Basidiomata stature, mature (left), young (right); B: Pileus surface, young (right), maturing (to the left); C: Lamellae and stipe color; young (right), mature (far left). Bar (A-C) = 10mm.

hollow and somewhat stiff; basal tomentum moderate to scarce, white at first, then yellowish. Bruising Reactions pileus, lamellae, and stipe none; basal tomentum at time becoming orangish.

Basidiospores isodiametric to heterodiametric, in profile and dorsiventral views, 5-angled, all angles usually distinct but at times distinctly angular on the basal end and obscurely angular or rounded at the apex, isodiametric and 5–6-angled in polar view, 6.2–8.3 × 5.2–7.5 µm (x = 7.5 ± 0.4 × 6.3 ± 0.5 µm; E = 1.04–1.40; Q = 1.19 ± 0.09 (subisodiametric); n = 59/2). Basidia clavate, hardly tapered at the base, full of granules just before spore production, 27.1–40.0 × 7.1–11.8 µm (x = 33.2 ± 3.4 × 9.3 ± 1.2 µm; E = 3.11–4.56; Q = 3.59 ± 0.34; n = 18/2); 4-sterigmate with the sterigma up to 8.0 µm long. Hymenial Cystidia absent. Lamellar tramal hyphae subparallel, in the center of the lamella mostly short to moderately long, a few long in the center of the lamellae, hyphae with rounded ends, 41.4–320.0 × 7.8–22.2 (n = 16/2). Pileipellis an entangled layer of hyphae composed of ± moniliform chains of 3–4 cells, the chains entangled, more or less erect on the disc, suberect and in clusters elsewhere with a cutis between the clusters, up to 400 µm thick on the disc, 205 µm near the disc. Pileocystidia present as the terminal cells of a chain of cells, consistently broad, clavate to cylindro-clavate, 33.7–111.7 × 9.8–21.3 µm (x = 15.0 ± 3.2µ wide; E = 2.55–6.80; Q = 4.13 ± 1.32; n = 16/2). Pileal tramal hyphae similar in shape but shorter and broader than the lamellar trama, 57.5–155.4 × 9.8–22.2 µm (n = 7/1). Stipitipellis a layer of hyphae similar in structure to pileipellis everywhere except at the apex where there are clusters of colorless caulocystidia with a few basidia present. Caulocystidia at the apex, colorless, in clusters, pyriform to broadly clavate to broadly cylindro-clavate 23.9–108.1 × 8.5–20.2 µm (E = 1.81–12.73); n = 16/2); elsewhere cylindro-clavate to clavate, similar in size, shape, and pigmentation as the pileocystidia. Oleiferous hyphae absent. Lipoid globules absent. Brilliant granules scattered. Pigmentation cytoplasmic and intracellular, brownish and insoluble in 3% KOH in the pileo- and caulocystidia; faintly parietal or incrusting in the lower portion of the pileipellis. Clamp connections present at the base of the basidia; absent in all other tissues.

Ecology & distribution — Typically gregarious, rarely scattered but rarely caespitose, on decomposing logs and in mosses in a subtropical gallery rainforest, Barrington Tops National Park, New South Wales. In April 2009 and 2010 several different populations were common on decaying logs in various localities towards the end of the Blue Gum Track in the Williams Day Use Area. Each population produced approximately 50–100 basidiomes typically in acrocarpous mosses resembling a species of *Dicranum* Hedw.

Additional collections examined — **AUSTRALIA. New South Wales**, central Hunter District, Barrington Tops National Park, Williams River Day Use Area, end of Blue Gum Track, 32°09′03.2″S 151°31′28.6″E, 8 April 2010, DL Largent 9847; 32°09′10.7″S 151°31′38.3″E, 14 April 2010, DL Largent 9872, 9881; 32°09′04.7″S 151°31′34.1″E, 18 April 2010, DL Largent 9899; 32°09′05.0″S 151°31′30.6″E, 20 April 2010, DL Largent 9914.

Plate 4. *Leptonia ambigua* {DLL 10130 holotype C-E; DLL 9872 A-B}. A: Basidiospores; B: Basidia and basidioles; C: Pileipellis and terminal pileocystidia with uniform brownish cytoplasmic pigment in 3% KOH; D: Parietal pigmentation in pileipellis; E: Stipitipellis at stipe apex with pyriform caulocystidia; F: Stipitipellis at stipe apex with clavate cheilocystidia. Bars: A = 5 µm; B = 10 µm; C-D = 15 µm; E = 10 µm.

Distinctive characters — Habitat on decomposing logs covered with mosses; convex dark greyish violet pileus that becomes brown with maturity; violet-white lamellae at first; dull violet stipe that becomes grayish violet with age; subisodiametric basidiospores, distinctly angular except at times obscurely angular at the apex; two types of caulocystidia at the stipe apex; the broad tramal hyphae in the lamellae; and no clamp connections in the pileipellis.

Comments — Its basidiome colors and stature, pileipellis type, and brownish cytoplasmic pigment cause *L. ambigua* to resemble several lignicolous species in *Leptonia* subg. *Leptonia*.

Entoloma obtusisporum E. Horak from Brazil also has obtusely angular basidiospores and lacks cheilocystidia but differs from *L. ambigua* by its white smooth stipe, lack of parietal pigmentation, and slightly smaller, isodiametric basidiospores that are 5.5–7.0 µm in all dimensions (Horak 1982). *Rhodophyllus dichrooides* Romagn. & Gilles from Gabon (Romagnesi & Gilles 1979), the European *L. dichroa* (Pers.) P.D. Orton (Noordeloos 1992), and *L. euchroa* (Pers.) P. Kumm. from Europe and Australia (Noordeloos 1992, May & Wood 1997) are differentiated by their heterodiametric angular basidiospores and abundant clamp connections in the pileipellis. *Leptonia tjallingiorum* (Noordel.) P.D. Orton from Europe is differentiated from *L. ambigua* by larger tricholomatoid basidiomes, abundant cheilocystidia, heterodiametric basidiospores, abundant clamp connections, and the absence of blue-black or dark violet colors in the pileus (Noordeloos 1992, 2004).

Leptonia omphalinoides Largent, **sp. nov.** Plates 5–6

MycoBank MB 802808

Distinct in the *Entolomataceae* by the lignicolous omphalinoid small basidiomes, small quadrate or cuboidal basidiospores, and clampless hyphae.

Type — Australia, northern Queensland, Mossman National Park, lower Track just past observation platform, within 20m of 16°28′17.6″S 145°19′51.7″E, 18 March 2010, DL Largent 9800 (**holotype**, BRI ; **isotype**, CNS).

Etymology — from the Greek ending "*-oides*" referring to the omphalinoid basidiome stature.

Pileus 6–11 mm broad, 2–4 mm high; opaque, not hygrophanous; convex to broadly convex, umbilicate; minutely tomentulose to scabrous and punctate on disc, minutely squamulose with the squamules erect towards the margin, appressed-fibrillose on the margin, striate to the disc, dull; disc reddish brown (8F4 to 8F7 'mahogany brown'), elsewhere reddish brown but a little lighter than the disc (8–9E-F6–7), eventually remaining dark brown on the disc but fading to greyish brown with a reddish tint elsewhere (8E-F3–4; margin decurved, minutely hairy then crenulate; context dark and less than 0.5 mm thick above the stipe. Odor indistinct. Taste indistinct. Lamellae up to

Plate 5. *Leptonia omphalinoides* {DLL9800 holotype A,B; DLL 9373 C}. A: Basidiomata stature; B: Pileal surface of young, dark basidiome; C: Pileal surface light form mature. Bar (A-C) = 2 mm.

12 per basidiome, 4–5 mm long, 1–1.5 mm deep, pallid greyish to off white when young, subdecurrent to decurrent, narrow, subdistant to distant; margin smooth and concolorous; lamellulae 1 to 3 between lamellae, in 1–2 tiers, 1 short or 2 short and 1 medium. Stipe 9–17 mm long, <1 mm broad, equal, glabrous, reddish grey to light dull red (8–9A1–2 or 8–9C2–3), hollow and very fragile; basal tomentum absent. Bruising reactions none.

Basidiospores consistently 4-angled, quadrate and small in all views, rarely cuboid, angles distinct and at times somewhat elongated and suggestively prismatic, isodiametric in polar views, subisodiametric to heterodiametric in profile and basal views, 4.6–8.4 × 3.7–6.7 µm (x = 6.1 ± 0.9 × 5.2 ± 0.7 µm; E = 1.00–1.52; Q = 1.19 ± 0.12 (subisodiametric); n = 54/2). Basidia clavate to subclavate, 21.5–34.8 × 7.2–11.3 µm (x = 28.4 ± 3.7 × 9.4 ± 1.0 µm; E = 2.2–4.1; Q = 3.09 ± 0.50; n = 24/2); base 3.1–5.1 µm; 4-sterigmate, sterigma 2.4–3.6 µm long. Cheilocystidia and Pleurocystidia abundant, particularly on the decurrent portion of the lamellae, and morphologically similar, colorless, versiform (cylindric, acicular, narrowly to broadly rostrate-ventricose), 27.3–49.1 × 4.3–13.0 µm (x = 41.0 ± 4.9 × 8.4 ± 2.2 µm; E = 5.20–10.19; Q = 5.20 ± 1.75; n = 21/2). Lamellar tramal hyphae relatively small, 37.6–88.1 × 2.4–14.1 µm (n = 12/2). Pileipellis 80–119 µm thick, badissima-type with laterally agglutinated, entangled hyphae but without collapsed cylindro-clavate cells, composed of only 1–3 cells; Pileocystidia broadly clavate to broadly cylindro-clavate, on the disc 14.3–96.3 × 4.0–23.1 µm (x = 56.5 ± 14.3 × 14.5 ± 4.0 µm; E = 1.2–6.8; n = 16/2), away from the disc 29.5–135.5 × 6.1–24.5 µm (x = 76.7 ± 29.5 × 17.2 ± 6.1 µm; E = 2.5–10.6; n = 11/1). Pileal tramal hyphae small, not very long, and so thin as to appear non-existent in sections, 50.3–63.7 × 9.5–11.9 µm; n = 3/1). Stipitipellis with hymenial elements at or next to decurrent lamellae, and otherwise a cutis. caulocystidia absent. Stipe tramal hyphae relatively small, 47.7–116.1 × 6.04–15.4 µm (n = 9/2). Oleiferous hyphae rare in the trama. Lipoid globules absent. Brilliant granules abundant in the basidia. Pigmentation very dark brown and in large granules to plaque-like areas in the pileipellis, suggestively parietal in pileal trama. Clamp connections absent in all tissues.

Ecology & distribution — Scattered on a single large decaying log, at the end of Marrdja Walk about 100 yards from the parking lot, Tribulation Section, Daintree National Park, and on piece of wood at base of a boulder on a ridge above an observational platform, lower river trail, Mossman National Park; mid-March.

Additional collections examined — **AUSTRALIA. Queensland,** Cook Region, Daintree National Park, Tribulation Section, 16°08′18.5″S 145°26′26.0″E, 20 March 2009, DL Largent 9673.

Distinctive characters — Small lignicolous omphalinoid basidiomes; striate pileus; badissima-type pileipellis lacking collapsed cylindro-clavate

Plate 6. *Leptonia omphalinoides* {DLL 9800 holotype E; DLL 9673 A – D}. A: Basidiospores; B: Basidia with globules; C: Obclavate cystidium; D: Basidium, cystidium becoming rostrate-ventricose, vesiculate cystidium; E: Pileipellis near disc, cylindro-clavate, clavate to broadly cylindro-clavate pileocystidia with dark brown cytoplasmic pigment in plaque-like areas. Bars: A = 6 µm; B-D = 10 µm; E = 20.0 µm.

pileocystidia; versiform hymenial cystidia; small 4-sterigmate basidia; dark brown plaque-like pigment in the pileipellis and suggestively parietal in the pileal trama; clampless hyphae; 4-sided subisodiametric basidiospores averaging <6.5 µm × <5.5 µm.

Comments — *Eccilia cubensis* Murrill from Cuba and *Entoloma brunneostriatum* Dennis from Trinidad also have small, omphalinoid basidiomes and small basidiospores. *Eccilia cubensis* differs from *L. omphalinoides* by its octahedral, larger basidiospores (7–9 µm), innately scaly pileus lacking striations, and slightly granular floccose stipe (Murrill 1911), while *Entoloma brunneostriatum* differs in its larger (7–8 × 6–8 µm) basidiospores, lack of hymenial cystidia, and smooth pileus and stipe (Dennis 1953).

Leptonia poliopus (Romagn.) P.D. Orton, Mycologist 5: 134 (1991). Plates 7–8

≡ *Entoloma poliopus* (Romagn.) Noordel., Persoonia 10: 262 (1979).

Pileus 9–22 mm broad, 1–2 mm high, dull; striate to the disc, suggestively translucent; depressed, convex then broadly convex; when young entirely tomentulose, with expansion and maturity remaining tomentulose on the disc but forming squamules from the disc to the margin, eventually appressed-fibrillose to nearly glabrous at or near the margin; at first greyish brown (6F3, 'negro') on the disc to a bit lighter brown elsewhere (6F3–4, between 'negro' and 'chocolate brown'), upon expansion retaining the greyish brown (6F3) color in the disc and in the striations but becoming dark brown (6F6, 'burnt umber' or 'vandyke brown') from near the center to the margin as the context shows through the areas between the squamules and the fibrils, hygrophanous between the marginal area and the center and thus becoming brownish orange to orange white (5 or 6C3 to 5–6A-B2–3); margin incurved to decurved then decurved, plane, and finally uplifted, even; context <1 mm deep, concolorous with the surface. Odor typically mild and indistinct, at times faintly fragrant. Taste typically mild to nearly bitter. Lamellae 4–10 mm long, 2–3 mm deep, broadly adnexed to adnate with a faint decurrent tooth or faintly subdecurrent, subdistant, moderately broad, pallid or off-white at first (5–6A2); margin in young and moderately aged basidiomes, smooth to the eye but at 10× minutely serrulate and thus cystidiate, typically concolorous, sometimes with a faint greyish line or with a distinct black to brown-black margin on some but not all lamellae, in mature specimens often appearing smooth when the marginal cystidia are collapsed. Lamellulae typically 3 (2 short, 1 moderately long) between lamellae, at times up to 7 (4 short, 2 moderately long, 1 long). Stipe 20–47 mm long, 1–2 mm broad at apex, 2–4.0 mm broad at base, subclavate, ± shiny, glabrous, at first dark violet or bluish grey (18–19F3) quickly becoming grey or dark brownish grey (5F1 to 6F2) but with a bluish grey tinge, glabrous, hollow, cartilaginous, relatively fragile; basal tomentum white, scarce to moderate. Bruising reactions absent.

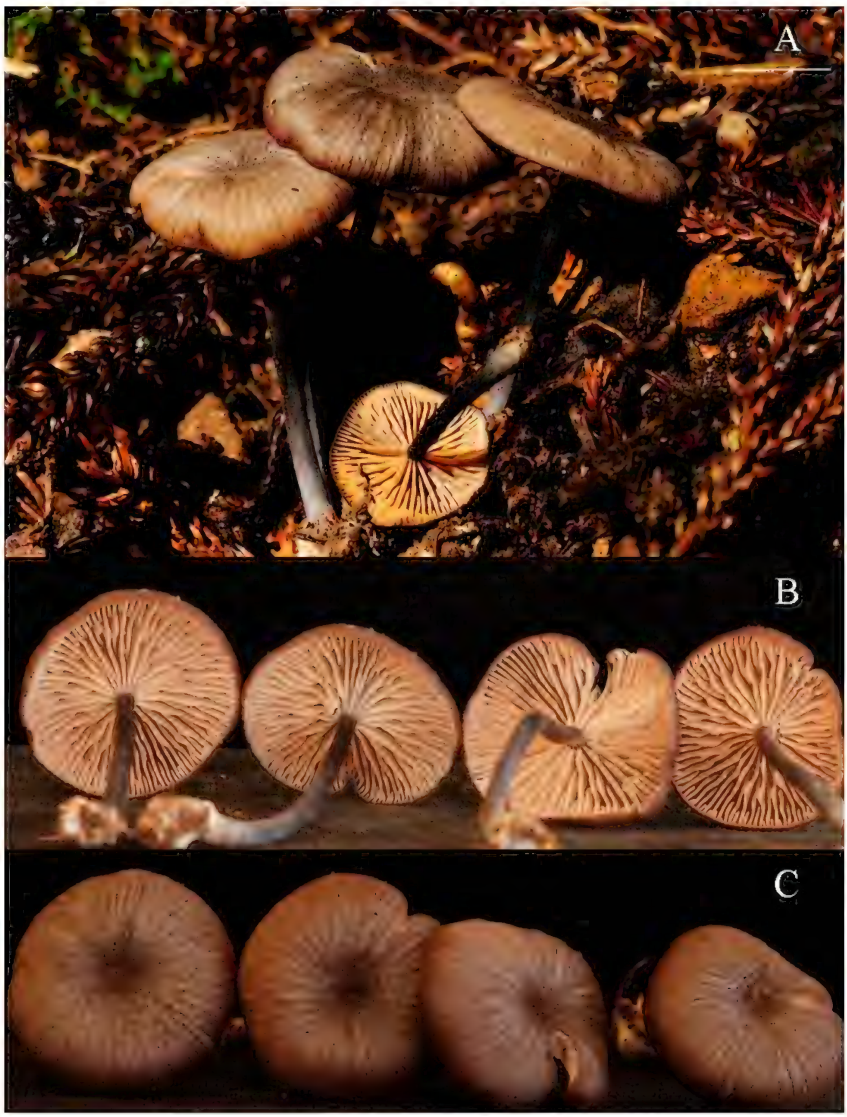


Plate 7. *Leptonia poliopus* {DLL 10209a)}. A: Basidiomata field (1.5); B: Lamellae ; C: Pileal surface. Bar (A-C) = 10 mm.

Basidiospores 5–6-angled, angles distinct, apex typically with a single rounded angle, nearly heterodiametric to typically heterodiametric in profile and dorsiventral view, 8.5–13.5 × 6.0–9.4 µm (x = 10.6 ± 1.0 × 7.8 ± 0.6 µm; E = 1.1–1.8; Q = 1.40 ± 0.12; n = 144/4). Basidia broadly clavate, hardly tapered,

with abundant granules prior to spore production, 25.9–44.0 × 7.9–13.1 µm (x = 33.4 ± 4.3 × 10.3 ± 1.2 µm; E = 2.54–4.10; Q = 3.27 ± 0.42; N = 48/3); 2 or 4-sterigmate, sterigma up to 6.0 µm long. CHEILOCYSTIDIA as terminal cells of branched hyphae in the hymenophore, abundant and forming a sterile layer, typically colorless but in 3% KOH with a brownish cytoplasmic pigment in specimens with marginate edges, broadly clavate to clavate, 27.2–67.1 × 10.7–20.9 µm (x = 41.6 ± 11.7 × 14.4 ± 2.7 µm; E = 2.9–5.0; Q = 2.9 ± 0.8; N = 26/3) or rarely cylindro-clavate and then 21.3–67.1 × 4.9–10.1 µm (x = 41.1 ± 10.5 × 7.9 ± 1.6 µm; E = 2.3–9.2; Q = 5.5 ± 2.0; N = 31/4), both clavate and cylindro-clavate cystidia present in same specimen (DLL 10209a and DLL 10209c) or cylindro-clavate entirely (DLL 10209b). PLEUROCYSTIDIA absent. LAMELLAR TRAMAL HYPHAE subparallel, with rounded to ± straight end cells, 76.9–291.1 × 5.7–19.1 µm; E 4.6–23.4; N = 12/2). PILEIPELLIS always a palisadoderm in the pileus disc, an entangled layer of hyphae with inflated terminal cells, at times with the terminal 2 to 3 cells inflated, 66–125 µm deep. PILEOCYSTIDIA in the disc broadly clavate, rarely nearly napiform, often long clavate, 33.6–99.4 × 14.2–29.9 µm (x = 54.8–15.1 × 20.4 ± 4.5 µm; E = 1.6–6.3; Q = 2.8 ± 1.1; N = 25/2). PILEAL TRAMAL HYPHAE similar to those of the lamellar trama, 91–272 × 4–20 µm; N = 6/1. OLEIFEROUS HYPHAE rare in the lamellar trama. LIPOID GLOBULES absent. BRILLIANT GRANULES absent. PIGMENTATION plasmatic, brownish in the pileipellis; absent to faintly brownish to brownish in the cheilocystidia. CLAMP CONNECTIONS absent in all tissues.

ECOLOGY & DISTRIBUTION — Several basidiomes frequently encountered in leaf humus or in bare soil under ferns or among rocks along the edge of the trail; basidiomes in one collection scattered, often gregarious. In early May 2011, numerous basidiomes (8–20 per collection) were found in Strickland State Forest in soil and humus and in protected areas near creeks, tracks, or roads.

ADDITIONAL COLLECTIONS EXAMINED — **AUSTRALIA. NEW SOUTH WALES**, central Hunter District, Strickland State Forest, Lower Parking Lot. Within 20 m of 33°22′45″S 151°19′32″E, 7 May 2011, DL Largent10209a, 10209b, 10209c.

DISTINCTIVE CHARACTERS — Greyish brown pileus, striate to the disc; pallid to off-white lamellae with concolorous or black to brown-black margins; bluish

TABLE 2. Comparison of macromorphology and micromorphology of *Leptonia poliopus* collections

COLLECTION	LAMELLAR MARGIN	BASIDIOSPORE (average)	
		LENGTH	WIDTH
Largent 10209a	Colorless, serrulate	11.0 µm	8.0 µm
Largent 10209b	Black, smooth	10.4 µm	7.6 µm
Largent 10209c	Black, serrulate	10.0 µm	7.5 µm

Plate 8. *Leptonia poliopus* {DLL 10209b A, C-E; DLL 10209c B}. A: Basidiospores; B: Cheilocystidia; C: Basidia; D: Pileocystidia; E: Palisadoderm on pileal disc. Bars: A= 5 µm; B-C = 15 µm; D = 10 µm; E = 25 µm.

grey to dark violet stipe that quickly becomes grey to brownish grey; 5–6-angled, heterodiametric basidiospores measuring 8.5–13.5 × 6.0–9.4 µm and averaging >10 µm × <8.0 µm; clavate to broadly clavate cheilocystidia; clampless hyphae.

Comments — Three collections of *Leptonia poliopus* were made in Strickland State Forest, each differing in the lamellar margin characteristics, type of cheilocystidia, and average basidiospore size (Table 2). As overall sequence similarity from all three loci was 100%, we conclude that these three collections represent the same taxon. The composite description above is drawn from the three collections and matches descriptions of European collections that may form concolorous or brown- marginate lamellar edges (Noordeloos 1992, 2004).

Entoloma asprellopsis G.M. Gates & Noordel. from Tasmania also shows the same lamellar margin variations but differs from *L. poliopus* in its blue-grey lamellae and slightly smaller (9–12 × 6–8 µm) basidiospores. *Entoloma griseosquamulosum* G.M. Gates & Noordel. differs by its smaller basidiospores, concolorous lamellar edges, a pileus margin that is opaque or only translucent-striate at the very edge, and a saliva-inducing taste (Noordeloos & Gates 2012). *Entoloma asprelloides* G. Stev. from New Zealand and *E. fuscomarginatum* (Cleland) E. Horak (nom. illegit.) and *E. fuscum* (Cleland) E. Horak from Australia all have similar-sized basidiospores but differ from *L. poliopus* by their concolorous pileus and stipe (Horak 1973, 1980). Additionally, *E. fuscomarginatum* (as "*rubromarginatum*") and *E. fuscum* lack cheilocystidia (Grgurinovic 1997). Other taxa with features similar to *L. poliopus* are discussed at the end of the descriptions for *E. saponicum*, *E. asprellopsis*, and *E. griseosquamulosum* (Noordeloos & Gates 2012),

Leptonia poliopus is not easy to differentiate from other species, in part because of the large number of morphologically similar species (Horak 2008: 186; Largent 1994: 146–152; Noordeloos 2008: 114; Noordeloos & Gates 2012: 287). Phylogenetic analyses should help to clarify species boundaries in future studies.

Leptonia umbraphila (Noordel. & Hauskn.) Largent, **comb. nov.** PLATES 9–10

MYCOBANK MB 802809

≡ *Entoloma umbraphilum* Noordel. & Hauskn., Fungal Diversity 27: 134 (2007).

PILEUS 8–25 mm broad, 2–4 mm high; opaque and not translucent nor hygrophanous; broadly convex to convex, umbilicate; tomentulose on the disc, squarrulose to appressed squamulose towards the margin, appressed fibrillose on the margin; at first disc very dark brown (7F2) elsewhere a bit lighter (7F4), then becoming dark brown (6–7F5–6), and eventually fading to medium brown (6–7E-F5–6), dull, striate nearly to the disc; margin incurved to decurved, striate, even becoming crenulate with age. TASTE distinctly farinaceous, at times latent. ODOR faintly but distinctly farinaceous. LAMELLAE 4.5–9 mm long, 1.25–5 mm high, adnate with decurrent tooth to subdecurrent, close to subdistant then distant in over-mature basidiomes, narrow to moderately broad, in some becoming broad, white to orange white to pallid (5A2) at first then more pinkish with basidiospore maturation; margin in places or entirely roughened to serrulate and distinctly brownish to blackish brown; 3 or 5–7 lamellulae between lamellae (in 2–4 rows, 2–4 short and 1–2 medium or 1 medium long). STIPE 8–30 mm long, 1.0–4.0 mm broad, equal, glabrous to faintly appressed fibrillose, a bit lighter than the pileus, medium brown (6–7E–F4–6), at times pale orange (5B3) at the apex, hollow and stiff but decays rapidly; basal tomentum scarce. BRUISING REACTIONS absent.

Plate 9. *Leptonia umbraphila* {DLL 9792 A; DLL 9793 B-C}. A: Basidiomata (3); B: Pileal surface (2.5); C: Marginate lamellar edge (2.5). Bar (A- C) = 10 mm.

Basidiospores typically cuboid in all views, at times 5-angled in dorsi-ventral view, angles distinct, mostly isodiametric but some may be heterodiametric, relatively small, 5.5–8.3 × 5.5–7.9 µm (x = 7.3 ± 0.50 × 6.7 ± 0.52 µm; E = 1.00–1.41; Q = 1.10 ± 0.09; n = 92/4). Basidia mostly 1–2-sterigmate, rarely 4-sterigmate, subclavate with the very base tapered, sterigma 2.1–4.8 µm; 22.5–40.6 × 6.5–11.0 µm (x = 28.6 ± 3.8 × 8.5 ± 0.9 µm; E = 2.8–4.5; Q = 3.4 ± 0.4; n = 40/3); base 1.6–4.5 µm. Cheilocystidia clavate, scattered to abundant, in clusters but not forming a sterile layer along the entire lamella, clavate, 23.3–53.5 × 5.1–10.7 µm (x = 37.5 ± 9.1 × 8.1 ± 1.8 µm; E = 2.3–7.9; Q = 4.8 ± 1.3; n = 16/2). Pleurocystidia absent. Lamellar tramal hyphae relatively slender and thus not broad, 40.8–144.1 × 2.2–22.0 µm (n = 26/3). Pileipellis badissima-type (laterally agglutinated hyphae abundant; slender cylindro-clavate collapsed on clavate to vesiculate pileocystidia), 80–135 µm

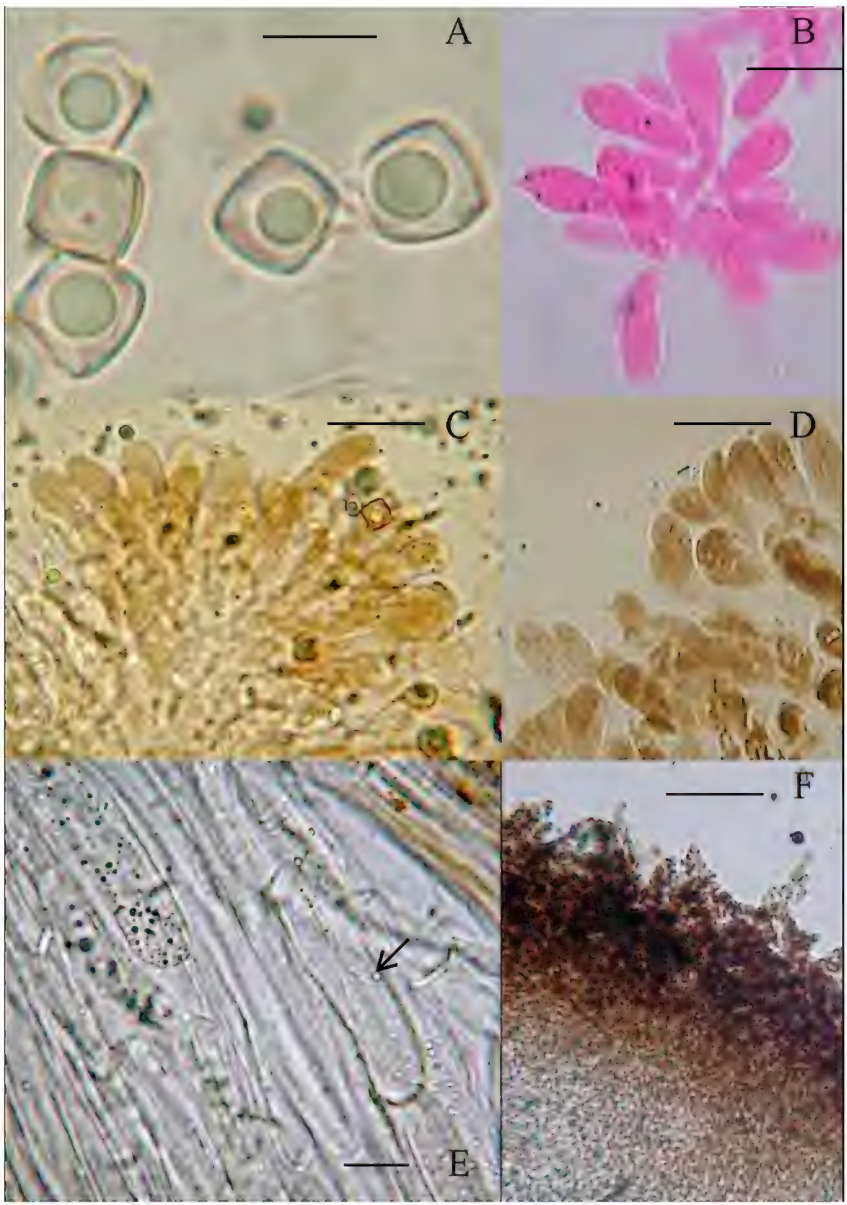


Plate 10. *Leptonia umbraphila* {DLL 9629 A-E; DLL 9792 F}. A: Basidiospores; B: 1-2 sterigmate basidia; C: Clavate cheilocystidia with brownish cytoplasmic pigment; D: Pileipellis, pileocystidia and cytoplasmic plaque-like pigment; E: Pileal trama with brilliant granules and parietal pigment (arrow); F: Pileipellis. Bars: A = 7 µm; B = 20µm; C = 30 µm; D = 20 µm; E = µm; F = 65 µm.

deep on the disc. Pileocystidia cylindro-clavate to clavate to vesiculate, 22.7–68.6 × 4.8–17.3 µm (x = 36.3 ± 12.9 × 10.8 ± 3.6 µm; E = 1.6–9.4; Q = 4.0 ± 2.4; N = 19/2). Pileal tramal hyphae relatively short, subparallel, 32.5–113.1 × 4.4–15.4 µm. Stipitipellis a cutis. Basidiole clusters rare, 29.7–44.8 × 8.2–17.0 µm (N = 8/1). Stipe tramal hyphae subparallel, 31.5–191.7 × 4.4–15.4 µm (N = 11/2). Oleiferous hyphae scattered in the stipe trama. Lipoid Globules absent. Brilliant granules abundant and small in the basidia and in the pileal tramal hyphae. Pigmentation pale brown, uniformly intracellular in the cheilocystidia; very dark brown, intracellular, and in pigment-globules in the hyphae of the pileipellis, not soluble in 3% KOH or in water. Clamp connections absent in all tissues.

Ecology & distribution — Scattered in bare soft soil in areas protected and shaded by a dense tree overstory, Mossman National Park; in bare compacted soil shaded by a single tree (but protected by roots) in middle of Emmagen Creek Trail, Daintree National Park; late February to late March.

Additional collections examined — **AUSTRALIA. Queensland**, Cook Region, Mossman National Park, 16°28′15.2″S 145°19′48.8″E, 27 February 2009, DL Largent 9629, 9630; 16°28′15.2″S 145°19′48.8″E, 9 March 2009, DL Largent 9640; 16°28′15″S 145°19′49″E, 4 March 2010, DL Largent 9766; 16°28′17.6″S 145°19′51.7″E, 18 March 2010, DL Largent 9792, 9793, 9799. Daintree National Park, Tribulation Section, 16°02′20.1″S 145°27′40.8″E, 22 March 2010, DL Largent 9816.

Distinctive characters —Small broadly convex-depressed dark brown pileus; weakly decurrent or subdecurrent lamellae; basidiospores cuboid or 5-angled, isodiametric, and averaging <8.1 µm × <7.5 µm; badissima-type pileipellis with dark brown, intracellular, pigment globules; basidia small, typically 2-sterigmate; clavate, cheilocystidia with pale brownish pigment.

Comments — The collections of *L. umbraphila* from northern Queensland are identical to the Seychelles holotype in habitat, all macrofeatures, and nearly all microfeatures. In the holotype (Noordel. & Hauskn. 2007 as *E. umbraphilum*), the basidiospores are a little larger (7–9 × 6.5–8.5 µm), the basidia are slightly smaller (23–31 × 8–11 µm), and the cheilocystidia somewhat smaller (20–30 × 8–11 µm) than in the northern Queensland collections. We consider these slight differences to be insignificant.

Rhodophyllus dicubospermus Romagn. & Gilles from Gabon closely resembles *L. umbraphila* with its omphalinoid stature, brown pileus and stipe, bisporic basidia, and cuboid basidiospores but differs in the reddish brown pileus, brownish white lamellae, concolorous lamellar margin, and slightly larger (6.5–9 × 6.2–8 µm) basidiospores (Romagnesi & Gilles 1979).

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Hymenochaetaceae from México 6. A new *Fuscoporia* species from the Sonoran desert

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Abstract — *Fuscoporia xerophila* is described as a new species from México. The collected specimens were found on dead or living wood in xerophilous scrub in the central plain of the Sonoran desert, Sonora State, México. This species is characterized by its resupinate basidiomata with medium size pores (4–5 per mm), its hymenial setae and hyphal setae, and basidiospores that are are subglobose, hyaline, smooth, and thin-walled. A key to Mexican species of *Fuscoporia* is presented.

Key words — *Agaricomycetes*, *Hymenochaetales*, polyporoid fungi, taxonomy

Introduction

Fuscoporia was described by Murrill (1907) with *F. ferruginosa* as the type species. Many mycologists (Overholts 1953, Lowe 1966, Ryvarden & Johansen 1980, Gilbertson & Ryvarden 1987, Larsen & Cobb-Poulle 1990, Ryvarden 1991) have regarded this genus as synonymous with *Poria* Adans. and *Phellinus* Quél. At the present time, Fiasson & Niemelä (1984), Wagner & Fischer (2001, 2002), Groposo et al. (2007), Baltazar et al. (2009), Baltazar & Gibertoni (2010), and Raymundo et al. (2013) recognize *Fuscoporia* as a monophyletic genus characterized by its annual to perennial and resupinate to pileate basidiomata with poroid hymenophore, its dimitic hyphal system with encrusted generative hyphae in the dissepiments and trama, hymenial setae that are always present and hyphal setae that are either present or absent, and basidiospores that are hyaline, non-amyloid, acyanophilous, subglobose, ellipsoid, cylindrical to allantoid, thin-walled, and smooth.

Recently, Raymundo et al. (2013) reported 14 *Fuscoporia* species from México: *F. callimorpha* (Lév.) Groposo et al., *F. chrysea* (Lév.) Baltazar &

Gibertoni, *F. contigua* (Pers.) G. Cunn., *F. contiguiformis* (Pilát) Raymundo et al., *F. coronadensis* (Rizzo et al.) Raymundo et al., *F. ferrea* (Pers.) G. Cunn., *F. ferruginosa* (Schrad.) Murrill, *F. gilva* (Schwein.) T. Wagner & M. Fisch., *F. mesophila* Raymundo et al., *F. nicaraguensis* Murrill, *F. palmicola* (Berk. & M.A. Curtis) Bondartseva & S. Herrera, *F. rhabarbarina* (Berk.) Groposo et al., *F. viticola* (Schwein.) Murrill, and *F. wahlbergii* (Fr.) T. Wagner & M. Fisch.

In 2009 several *Fuscoporia* specimens were collected during a survey of Sonoran desert in Sonora State, México, that did not represent any known species of *Fuscoporia*. They are described here as a new taxon.

Materials & methods

The Sonoran desert covers 223,009 km^2, of which 29% lies in Arizona and California, U.S.A., and 71% in State of Sonora, México (Robles-Gil et al. 2001). It presents a moderate biological richness with xerophilous scrub and dry or very dry weather. The examined specimens were collected in Sonora State in August, 2009, and are deposited in the Herbarium, Instituto Politécnico Nacional, México (ENCB) with an isotype deposited in the fungal collections of the of the Universidad Estatal de Sonora, México (UES). Longitudes and latitudes were obtained with GPS eTrex (Garmin). Herbaria acronyms follow Thiers (2012) and colors are coded according to Kornerup & Wanscher (1978). Morphological examinations were conducted using protocols outlined by Cifuentes et al. (1986) and Ryvarden (1991). Anatomical characters were measured from rehydrated tissues in 5% aqueous KOH and Melzer's reagent. Line drawings were made to scale using an optical light microscopy with clear camera and macroscopic photographs were taken with a Nikon Coolpix 4300. The meanings of some terms are based on Ulloa & Hanlin (2012).

Taxonomy

Fuscoporia xerophila Raymundo, R. Valenz. & Esqueda, **sp. nov.** FIGS 1–9

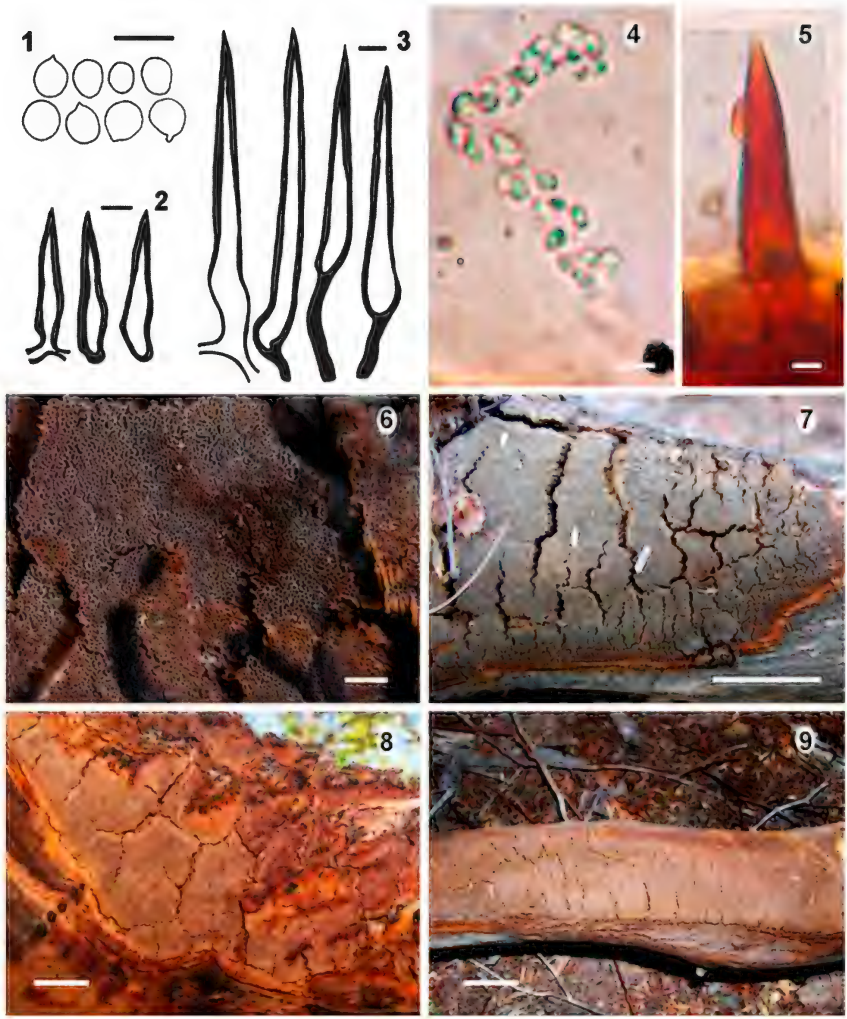
MycoBank MB801799

Differs from *Fuscoporia contigua* by its smaller pores, its shorter and wider subglobose basidiospores, and its xeric habitat.

TYPE: México, Sonora, municipality of Guaymas, Francisco Villa "El Tigre", 28°06′56.3″N 111°01′35.2″W, alt. 140 m, 21.VIII.2009, R. Valenzuela 13772 (**Holotype**, ENCB, **isotype**, UES).

Etymology: Referring to its occurrence in xerophilous scrub with very dry weather.

Basidiomata annual to perennial, resupinate, adnate, becoming widely effused, reaching 300–600 × 80–150 × 1–3 mm, corky to woody, easily separable of the substrate, cracked in dry and old specimens. margin sterile, ≤3 mm wide, golden brown (5D7), yellowish brown (5F7) to grayish brown (6E3), matted, fimbriate. hymenophore poroid, cracked with age or in dry specimens, grayish brown (6D–E3) to brown (6E4–5), dark brown (6F6) in old specimens; pores angular to irregular, (3–)4–5 per mm, 200–240 × 144–176 μm (av. 217



Figs 1–9. *Fuscoporia xerophila* (1-7,9—holotype, ENCB Valenzuela 13772; 8—ENCB Raymundo 3233): 1. Basidiospores. 2. Hymenial setae. 3. Hyphal setae. 4. Generative hyphae strongly incrusted with crystals. 5. Hymenial setae. 6–9. Basidiomes. Scale bars: 1–3 = 8 μm; 4–5 = 5 μm; 6 = 2 mm; 7–9 = 100 mm.

× 160 μm); dissepiments thin to thick and lacerated, (48–)80–140(–160) μm thick, (av. 112 μm); tubes ≤1 mm deep, 1–3 layers, tough to woody, grayish brown (6E3) to brown (6E5). context or subiculum very thin, <1 mm thick, yellowish brown (5F7), fibrous, tough, azonate.

Hyphal system dimitic, generative hyphae simple-septated, hyaline to pale yellow in KOH, simple to slightly branched, thin-walled, 2.4–3.2 µm in diam.; skeletal hyphae yellowish brown to reddish brown, unbranched or rare branched, thick-walled, 3.2–4.8 µm in diam. hymenophoral trama composed by interwoven hyphae, generative hyphae hyaline to pale yellow in KOH, thin-walled, simple to slightly branched, 2.4–3.2 µm in diam., hyphae projecting beyond the hymenia and in the edge of dissepiments strongly encrusted with crystals; skeletal hyphae yellowish brown to reddish brown in KOH, unbranched, thick-walled, 3.2–4.8 µm in diam. contextual trama with interwoven hyphae, generative hyphae rare, hyaline to pale yellow in KOH, simple to scarcely branched, thick-walled, 1.6–2.4 µm in diam.; skeletal hyphae dominant, reddish brown in KOH, unbranched, thick-walled, 2.4–4.8 µm in diam. hyphal setae 88–180 × 12–20 µm, straight, lanceolate to lageniform, acute, dark brown in KOH, thick-walled, only found in subiculum and margin. hymenial setae 40–52 × 6.4–8 µm, ventricose to ventricose-rostrate, acute, reddish brown to dark brown in KOH, thick-walled. basidia not observed. basidiospores 5.12–5.46 × 4–4.48 µm, subglobose, hyaline in KOH, inamyloid, thin-walled, smooth.

Ecology & distribution— The species grows on live and dead wood of *Acacia* in xerophilous scrub, causing a white rot. It is known only from the type locality in Sonoran desert in México.

Additional specimens examined: **MÉXICO, SONORA**, municipality of Guaymas, Francisco Villa "El Tigre", 28°06′56.3″N 111°01′35.2″W, alt. 140 m, 21.VIII.2009, T. Raymundo 3229, 3230, 3232, 3233 (ENCB); R. Valenzuela 13771, 13773 (ENCB).

Comments— The species is characterized by its resupinate basidiomata with angular to irregular pores, hyphal setae present in subiculum and margin, and subglobose basidiospores. *Fuscoporia contigua* is similar to *F. xerophila* but has larger pores (1–3 per mm), hyphal setae in the floccose margin, tramal and hymenial setae in the trama, and longer oblong to subcylindrical (5–7 × 3–3.5 µm) basidiospores. Further, *F. contigua* grows on dead wood in tropical forests in México and the Neotropics (Ryvarden & Guzmán 1993, Ryvarden 2004), on *Quercus* in China (Dai 1999), and on bamboo in Brazil (Coelho et al. 2009). Other related species are *F. ferruginosa* and *F. nicaraguensis*, which have resupinate basidiomata and hyphal setae in the margin or trama. Further, both have small circular pores (7–9 per mm) and grow on dead wood of tropical forests; *F. ferruginosa* has longer cylindrical (5–7 × 2.4–3.5 µm) basidiospores while *F. nicaraguensis* has smaller globose (2.4–4.4 µm in diam.) basidiospores (Larsen & Cobb-Poulle 1990, Raymundo et al. 2013).

Key to *Fuscoporia* species in México

1. Hyphal setae present in trama, margin and/or subiculum **2**
1. Hyphal setae absent **5**

2. Basidiospores globose to subglobose, basidiomata resupinate 3
2. Basidiospores oblong to cylindrical, basidiomata resupinate to pileate 4
3. Pores 7–9 per mm, hymenial setae 24–36 × 6.4–9.2 µm, subulate, straight and hyphal setae in the sterile margin 60–136 × 6–12 µm, basidiospores 2.4–4.4 µm, globose ..... ***F. nicaraguensis***
3. Pores 4–5 per mm, hymenial setae 48–52 × 6.4–8 µm, ventricose to ventricose-rostrate, straight; hyphal setae in the margin or subiculum 88–180 × 12–20 µm, straight, lanceolate to lageniform; basidiospores 5.12–5.46 × 4–4.48 µm, subglobose ..... ***F. xerophila***
4. Pores 1–3 per mm, hymenial setae 40–60 × 6–8 µm, subulate, straight, tramal setae 50–90 × 5–12 µm, subulate, straight and hyphal setae in the floccose margin 80–120 × 6–12 µm, basidiospores 5–7 × 2.4–3.5 µm, subcylindrical to oblong ..... ***F. contigua***
4. Pores 7–9 per mm, hymenial setae 30–60 × 6–10 µm, subulate, straight and hyphal setae in the sterile margin 90–150 × 6–10 µm, basidiospores 5–7 × 2.4–3.5 µm, cylindrical ..... ***F. ferruginosa***
5. Basidiomata resupinate to effused-reflexed 6
5. Basidiomata pileate-sessile 9
6. Pores 1–3 per mm 7
6. Pores 4–6 per mm 8
7. Basidiospores oblong to cylindrical, 4–5 × 2–2.5 µm, pores angular, sinuous to labyrinthiform, hymenial setae 32–64 × 6.4–7.2 µm, subulate ..... ***F. palmicola***
7. Basidiospores cylindrical to slightly curved, 7–8 × 1.6–2 µm, pores angular, hymenial setae 50–70 × 5–7 µm, subulate ..... ***F. contiguiformis***
8. Basidiospores broadly ellipsoid to subglobose, 3.2–4 × 2.4–3 µm, hymenial setae 30–50 ×5–8 µm, ventricose to subulate ..... ***F. chrysea***
8. Basidiospores cylindrical, 5.5–7.6 × 2.4–3.2 µm, hymenial setae 27–37 × 5–7 µm, subulate ..... ***F. ferrea***
9. Basidiospores cylindrical to slightly curved, 7–9 × 1.5–2 µm, hymenial setae 25–75 × 5–8 µm, subulate ..... ***F. viticola***
9. Basidiospores globose to subcylindrical, never curved and >2 µm in diameter **10**
10. Hymenial setae hooked, some straight, 25–44 × 8–13.2 µm, basidiospores subglobose to ellipsoid, basidiomata applanate, dimidiate to broadly attached, pores 6–8 per mm, circular to elongate ..... ***F. wahlbergii***
10. Hymenial setae straight **11**
11. Basidiomata large, 100–350 × 150–300 × 25–100 mm, applanate, dimidiate to broadly attached, pores (5–)6–7(–8) per mm, circular, hymenial setae 20–32 × 6.4–9 µm, ventricose to subulate, basidiospores 3.2–5.6(–6) × 3.2–4 µm, subglobose to ovoid, growing only on conifers ..... ***F. coronadensis***
11. Basidiomata small to medium size, growing only on angiosperms **12**

12. Pileus surface with a distinctive black crust, glabrous and sulcate, pores 6–9 per mm, hymenial setae 20–30 × 4.8–7.2 µm, subulate, basidiospores 3.2–4 × 2–3 µm, ellipsoid ..... *F. rhabarbarina*

12. Pileus surface without a distinctive black crust **13**

13. Basidiomata triquetrous to ungulate, pileus surface velutinate to tomentose, concentrically sulcate, pores 6–8 per mm, circular, hymenial setae 28–40 × 8.8–16 µm, ventricose, basidiospores 4.0 × 3.2–3.8 µm, subglobose to ovoid ..... *F. mesophila*

13. Basidiomata applanate to convex, hymenial setae <10 µm broad **14**

14. Hymenial setae 16–28 × 6–8.8 µm, subulate to ventricose, basidiospores 3.6–4.8 × 2–3 µm, oblong to subcylindrical ..... *F. callimorpha*

14. Hymenial setae 20–40 × 6–10.2 µm, ventricose to subulate, basidiospores 4–5 × 2.5–3 µm, narrowly ellipsoid to oblong ..... *F. gilva*

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Phallus hadriani and *P. roseus* from Pakistan

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Abstract — Specimens of *Phallus hadriani* and *P. roseus* collected in Pakistan are described here. Photographs and molecular data are provided for both species.

Key words — *Basidiomycota, Phallales,* taxonomy.

Introduction

The genus *Phallus* Junius ex L. (*Phallaceae* Corda, *Phallales* E. Fisch.) is typified by *P. impudicus* L. Kreisel (1996) has recognized 31 different species within *Phallus* sensu lato (incorporating *Aporophallus*, *Dictyophora*, *Echinophallus*, *Endophallus*, *Itajahya*, and several other genera). Calonge (2005) proposed a provisional key for 25 species, after which several new species have been described (e.g., Calonge et al. 2005, 2008; Li et al. 2005; Moreno et al. 2009).

Phallus has a cosmopolitan distribution (Kreisel 1996); four species previously recorded from Pakistan include *P. celebicus* Henn., *P. impudicus*, *P. rubicundus* (Bosc) Fr. (Ahmad 1952), and *P. calongei* G. Moreno & Khalid (Moreno et al. 2009). Descriptions for two additional species based on recent collections from Pakistan are provided below.

Materials & methods

The collections preserved in LAH herbarium were examined and divided, with separate vouchers being deposited in AH herbarium. Specimens were mounted in Hoyer's medium and studied with a Nikon microscope. Spores were measured under the oil immersion objective.

DNA was extracted and amplified as described in Alvarado et al. (2012). The primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) were used to amplify the

Fig. 1. *Phallus hadriani:* Mature fruiting bodies with an egg. Bar = 0.5 cm

internal transcribed spacer region. Sequences were visually inspected for reading errors in MEGA4.0 (Tamura et al. 2007). Validated sequences were stored in GenBank under the accession numbers listed below. These sequences were aligned with the closest matches obtained with BLAST searches through the public databases. ITS sequences came mainly from Cheong (unpublished). Sequences were first aligned in MEGA 4.0 software using its ClustalW application and then corrected manually. The aligned locus was loaded in PAUP* 4.0b10 (Swofford 2001) and a maximum parsimony phylogenetic tree was reconstructed (2000 bootstrap replicates, TBR swapping algorithm, 50 sequence additions per replicate, MulTrees not in effect). It was also subjected to MrModeltest 2.3 (Nylander 2004) in PAUP* 4.0b10. The best model was then implemented in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where a Bayesian analysis was performed (5.8S-ITS2 data partitioned, 2 simultaneous runs, 6 chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 200,000 generations. Significance thresholds were above 70% for bootstrap (BP) and 99% for posterior probability (PP).

Taxonomy

Phallus hadriani Vent., Mém. Inst. nat. Sci. Arts 1: 517 (1798) FIG. 1

EGG ≤50 mm tall × 30 mm diam., subglobose to ovoid, sheathed by a tough, brown membrane. MATURE FRUITING BODY grayish to violet, ≤123 mm high, attached to the substratum by well-developed thick branched rhizomorphs at the base. RECEPTACULUM with an off white reticulate pileus head and stipe. PILEUS slightly campanulate, having glebal mass, ≤30 mm tall × 25 mm diam., cylindrical, narrowing at the top, hollow. GLEBA olivaceous green wet, sticky, deliquescent, not very smelly. STIPE off white, spongy, hollow, porous, ≤80 mm tall × 17–20 mm diam., cylindrical, almost equal, firm, hard. VOLVA thick, texture soft, cup-shaped, covering 1/3rd of the stipe, globose, grayish to violet with off white tones, outer surface relatively smooth, not encrusted with soil particles, lined with grayish to violet gelatinous and sticky material inside, ≤37 mm tall × 35 mm diam. SPORES 3–5 × 1-2 µm, ellipsoid, hyaline, smooth.

MATERIAL EXAMINED: **PAKISTAN**: KHYBER PAKHTUN KHWA, Khanspur, near P.U. teachers suites, ca. 2250 m a.s.l., solitary, on ground, on mountainous slopes, in Himalayan moist temperate forest, 22 Aug. 2010, N. Yousaf NYG01 (LAH 228210; AH 39161, GenBank KF481956).

Phallus roseus Delile, Descr. Egypte, Hist. Nat. 2: 300 (1813) FIGS 2–6

≡ *Itajahya rosea* (Delile) E. Fisch., Ber. Deutsch. Bot. Ges. 47: 294 (1929)

EGG off white to pinkish, subglobose, outer surface rough, encrusted with soil particles, 55 mm tall × 75 mm diam.; longitudinal section showing three layers forming a semicircle: outer layer 6mm thick, grayish with off white tones; middle layer ≤9 mm wide, grayish, smooth, shiny, wet, sticky, inner layer brownish, roughened by finger-like projections facing the centre; core white, soft, covered by layers on all sides except at the base. MATURE FRUITING BODY

Figs 2-6: *Phallus roseus:* 2. Eggs of fruiting body. 3. Cross-section of the egg showing three-layered arrangement. 4–5. Fruiting body development. 6. Mature fruiting body. Bars = 20 mm.

gregarious, ≤80 mm tall × 17 mm diam at the top and 45 mm at the base, attached to the ground by well-developed, branched, white, thick rhizomorphs. Receptaculum bearing pileus and stipe. Pileus globose to subglobose, 21 mm high × 34 mm diam., hollow, having glebal mass with irregular appendices pinkish. Gleba grayish, turning blackish with age, unpleasant odor when collected. Soft, wet, sticky. Stipe off pinkish white to pinkish, hollow, spongy, porous, hard, entirely enclosed by volva, broader and wider at the base

(30 mm in diam.) and narrowing upwards (25 mm diam.), some stipe flesh present at the top of receptaculum as an extension, extended part irregularly shaped, pinkish, ≤17 mm high. Volva ovoid, saccate, 45 mm tall × 30–45 mm diam., prominent, well developed, covering all the stipe almost from all sides, ends where glebal mass is present, whitish, thick, texture soft, outer surface rough, slightly having patchy appearance, encrusted with soil particles, smooth, membranous, gelatinous and shiny from inside, remnants of volva present on the stipe in form of white patches. Spores 3–4 × 1.5-2 µm, ellipsoid, hyaline, smooth.

Material examined: **PAKISTAN**: Punjab, Lahore, University of the Punjab, Quaid-e-Azam campus, Plant nursery, RO II, ca. 217 m a.s.l., gregarious, on ground, under *Dalbergia sissoo* Roxb. (*Fabaceae*), 10 Aug. 2010, N. Yousaf NYG02 (LAH 108210, GenBank KF481955; AH 39160).

Discussion

Phallus hadriani, currently included in *Phallus* sect. *Dictyophora* (Desv.) Kreisel, is characterized by its reticulate pileus surface with pinkish or violet tones. It somewhat resembles *P. impudicus*, a species with a whitish volva that lacks the purple or pinkish purple tones typical of *P. hadriani*. Kreisel (1996)

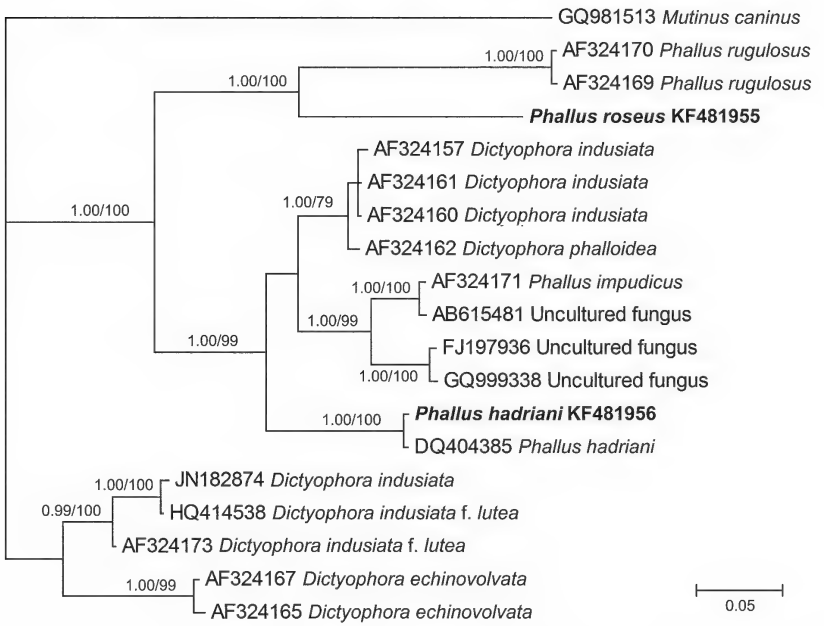

Fig. 7: Consensus ITS tree generated in MrBayes 3.1 showing the examined specimens and their closest relatives in the *Phallaceae*. Values above nodes represent PAUP maximum parsimony bootstrap proportions, while those below nodes represent MrBayes posterior probabilities.

cites *P. hadriani* from temperate and Mediterranean Europe, central Asia, China, Japan, and North America.

Although Fischer (1929) transferred *P. roseus* to *Itajahya,* Kreisel (1996) includes the species in *Phallus* subg. *Itajahya* (Möller) Kreisel, together with *P. galericulatus* (Möller) Kreisel and *P. glutinolens* (Möller) Kuntze (emend. Trierveiler-Pereira et al. 2009). *Phallus glutinolens* can be separated from both *P. roseus* and *P. galericulatus* based on its pinkish stipe and greenish gleba; *P. galericulatus* does not present a greenish gleba. *Phallus roseus,* a rare species from desert areas, was recently recorded from the Hadramout valley in Yemen (Kreisel & Al-Fatimi 2008) and the semi-arid Brazilian region (Ottoni et al. 2010).

We provide the first molecular data for *P. roseus*, which clearly separate the species from the *impudicus/hadriani* group and apparently link it to *P. rugulosus* Lloyd. Additionally, molecular data suggest that a cryptic species could exist within *P. impudicus*, as shown by the different lineages observed in the phylogenetic tree. However, the lack of sequences coming from available fruiting bodies make necessary further sampling efforts to confirm this hypothesis.

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Nidula shingbaensis sp. nov., a new bird's nest fungus from India

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Abstract —A new species of bird's nest fungi, *Nidula shingbaensis,* is proposed from the state of Sikkim. It is characterised by a slightly flared moderate to large peridium, yellowish interior peridium-wall, numerous brown-coloured peridioles with irregularly wrinkled surfaces, large broadly ellipsoid to elongate basidiospores, and a six-layered (in cross-section) peridium. A detailed description is supported by macro- and micromorphological illustrations, and the relation with similar and related taxa is discussed.

Key words — *Basidiomycota*, macrofungi, *Agaricaceae*, *Agaricales*, taxonomy

Introduction

Bird's nest fungi, previously placed in a separate family *Nidulariaceae*, were recently moved to the *Agaricaceae* (Kirk et al. 2008). Currently, they are represented in India by three genera with 17 species (14 *Cyathus* spp., *Nidula emodensis*, *N. candida,* and one *Crucibulum* sp.; Das & Zhao 2012).

Shingba Rhododendron Sanctuary (43 km^2) lies in the North district of Sikkim (a small Indian state in the eastern Himalaya). This subalpine area in the Yumthang valley and surroundings is covered by over 40 *Rhododendron* species but otherwise dominated by trees (*Abies densa*, *Picea spinulosa*, *Tsuga dumosa*, *Larix griffithii*, *Magnolia globosa*, *M. campbellii*, *Acer pectinatum*, *Betula utilis*) and harbours abundant macrofungi. During a 2011 macrofungal survey, the senior author came across a striking bird's nest fungus, which, after thorough macro- and micromorphological studies and literature surveys, we propose here as a new species, *Nidula shingbaensis*. We offer a detailed description and illustrations, discuss the relationship of the new species with allied Indian and extralimital taxa, and provide a provisional key to all species of *Nidula*.

Materials & methods

Macromorphological characters were recorded from the fresh basidiomata in the field and in the base camp. Field photographs of the fresh basidiomata were taken with the aid of Nikon D300s. Colour codes and terms (mostly) are as in the Colour identification chart of British Fungus Flora (Henderson et al. 1969). Immediately after recording the macromorphological characters, basidiomata were dried with a field drier.

In the laboratory, macromorphological characters were again observed from the dry samples with the help of stereo zoom dissecting microscope Nikon SMZ 1500 and photographs were taken through the attached dedicated camera. Other micromorphological structures such as basidiospores, cross-sections of peridium and peridiole were noted with the aid of light microscope Nikon E 50i and photography was made with the attached dedicated camera based on the free-hand sections from dry samples mounted in a mixture of 5 % KOH, 30 % glycerol, phloxine and cotton blue. Spore measurements are noted based on twenty basidiospores. Spore size measurements and length/width ratios (Q) are presented as: minimum–**mean**–maximum. Herbaria names are after Holmgren et al. 1990.

Taxonomy

Nidula shingbaensis K. Das & R.L. Zhao, **sp. nov.** PLATES 1–2

MYCOBANK: MB 803499

Differs from all other *Nidula* species by its six-layered peridium.

TYPE: India. SIKKIM: North District, Shingba Rhododendron Sanctuary, 27°43′28.1″N 88°44′53.8″E, alt. 2997 m, 24.VIII.2011, K. Das, KD-11-76 (**Holotype**, BSHC; GenBank KC763972. **Isotype**, SWFC).

ETYMOLOGY: after Shingba Rhododendron Sanctuary, the type locality

BASIDIOMATA urn- or vase-shaped, 6–9 mm high (≤11 mm high with epiphragm/lid), 5–7 mm wide at mouth, gradually tapering towards base (up to 4 mm wide), sessile. EPIPHRAGM operculate, buff (52), rupturing at maturity. EXTERIOR of the peridium distinctly matted fibrillose to villose, or wooly, hairs mostly in tufts (≤230 μm high), white to buff or clay pink; mouth slightly flared, margin straight to recurved, fringed, white (1A1), never wooly, never split into stellate lobes. INTERIOR of the peridium smooth (never plicate), never shining, saffron (49) to pale yellow (3C) towards mouth, rust or darker towards base. PERIDIOLES numerous (≤40), 0.9–1.3 mm in diam., lenticular, surface irregularly wrinkled, very sticky, embedded in transparent mucilaginous gel when moist, cinnamon (10), snuff brown (17) to cigar brown (16); funiculus absent.

PERIDIUM 650–720 μm thick (excluding hair), comprising six layers. Four outer layers composed of loose hyphal mats, "intricata" textura; each layered separated by a cord of grouped hyphae; the four (from the outermost) are 210 μm, 80 μm, <85 μm, and <120 μm thick; hyphae 3.5–5 μm wide, thick-walled (wall ≤2 μm thick), with clamp connections, hyaline to greenish yellow in KOH.

Plate 1: *Nidula shingbaensis* (Holotype KD-11-76). A, E. Basidiomata attached on substrates showing epiphragm and peridioles; B. Exterior of peridium (from dry sample); C. Peridium showing flared mouth and peridioles; D. Peridioles with wrinkled surface; F. Longitudinal section of peridium showing the interior (from dry sample). Scale bars: B, C, F = 1 mm; D = 0.5 mm.

Two inner layers of very similar nature, comparatively compact, placed above the internal (inner most) edge of disintegrated hyphae; the outermost is <90 µm and the innermost <190 µm thick. Hairs (≤70 µm wide at base) originating

from hyphal cord, passing through two or three outer layers projected beyond (emergent) the outer most layer ≤230 μm. Peridioles three layered (cortex, subcortex and hymenium), covered with tunica (≤27 μm thick); cortex ≤48 μm thick, two layered, consisting of an exocortex with hyphal tips (hyphae ≤2.2 μm wide) projecting in tunica, blue (72) in cotton blue and an endocortex with branched aseptate slightly to very thick-walled (≤4 μm thick) grayish yellow to rust (13) or rusty tawny (14) hyphae (≤10 μm wide); subcortex thick (≤120 μm), yellowish brown to cinnamon in cotton blue, subhyaline towards hymenium, with very thick-walled (wall ≤8 μm thick) hyphae having narrow lumen, bluish with cotton blue; hymenium ≤145 μm thick, subhyaline, blue in cotton blue, containing spore mass and hyphae (≤2 μm wide). Capillitium absent. Basidia not found (in mature peridioles). Basidiospores 6.9–**8.3**–9.8 × 4.9–**5.4**–6.1 μm, broadly ellipsoid to elongate (Q = 1.25–**1.52**–1.88), mostly rounded at both ends or at least at one end, thick-walled (≤1 μm thick), subhyaline, contents blue in cotton blue, hyaline to greenish yellow (inamyloid) in Melzer's reagent and pink in phloxine.

Ecology & Distribution — Gregarious to caespitose on small fallen twigs of *Abies densa* Griff. in subalpine coniferous or mixed forest. August. Rare.

> Additional specimen examined: **India**. **Sikkim**: North District, Shingba Rhododendron Sanctuary, 27°43′48.6″N 88°44′32.0″E, alt. 3095 m, 25.VIII.2011, K. Das, KD-11-92 (BSHC).

Notes — Its morphological characters (urn- to vase-shaped basidiomata containing numerous lenticular brown peridioles, six-layered peridium, mouth with epiphragm/lid, presence of tunica on the peridioles, two-layered cortex enclosing basidia and basidiospores, absence of capillitium and presence of smooth, hyaline, broadly ellipsoid to elongate basidiospores) easily place the present species amongst bird's nest fungi. Moreover, the presence of a multi-layered peridium with epiphragm/lid, absence of funiculus, and presence of transparent mucilaginous gel surrounding the peridioles confirms its placement in *Nidula* (Brodie 1975; Miller & Miller 1988).

Nidula shingbaensis is distinguished by its moderate to large peridium (6–9 × 5–7 mm), yellowish interior peridium-wall, brown-coloured large (0.9–1.3 mm diam.) peridioles with irregularly wrinkled surface, broadly ellipsoid to elongate large (6.9–9.8 × 4.9–6.1 μm) basidiospores, and six-layered peridium (in cross-section).

Macroscopically the new species is quite similar to three other *Nidula* species: *N. emodensis* (Berk.) Lloyd (Berkeley 1854; Cunningham 1924), *N. candida* (Peck) V.S. White (Brodie 1975; Das & Zhao 2012) and *N. macrocarpa* Lloyd (Lloyd 1917). However, *N. emodensis* differs in its smaller (4–6 mm high) peridia and relatively small (0.5–1 mm diam.) peridioles that are almost black at maturity (Cunningham 1924). *Nidula candida* differs by the larger peridium

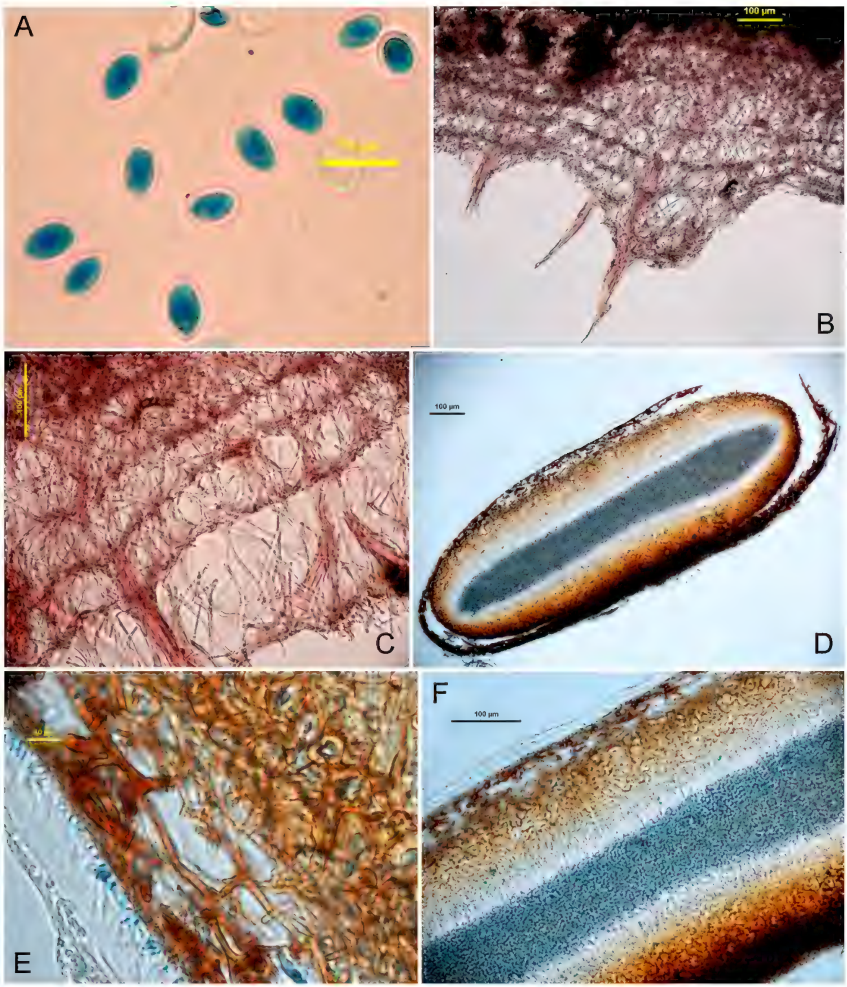

Plate 2: *Nidula shingbaensis* (Holotype KD-11-76). A. Basidiospores; B. Cross-section of peridium showing six layers; C. Four loose outer layers of peridium; D, F. Cross-section of peridiole showing tunica, cortex, subcortex and hymenium (blue), E. Transverse-section of peridiole showing tunica covering two layered cortex and subcortex. Scale bars: A, E = 10 µm; B–D, F = 100 µm.

(8–13 mm high) with more flared mouth and larger peridioles (1.5–1.8 mm diam.; Das & Zhao 2012). Similarly, *N. macrocarpa* (reported from Argentina and Chile) can be separated from *N. shingbaensis* by its small (0.5–1 mm diam.) peridioles and single layered peridium in cross-section (Diehl 2000). Finally, another species, *N. niveotomentosa* (Henn.) Lloyd (reported from North

America), differs from *N. shingbaensis* by its shorter peridium (5–7 mm high) and smaller peridioles (0.5–1 mm diam.; Miller & Miller 2006).

A provisional key to *Nidula* species

1. Basidiomata large, often ≥ 10 mm high 2
1. Basidiomata small, always <10 mm high 3
2. Peridioles 1.5–3 mm diam.; basidiospores 8–10 × 4–6 µm ..... *N. candida*
2. Peridioles 0.5–1.0 mm diam.; basidiospores 12–16 × 5–6 µm ..... *N. macrocarpa*
3. Exterior of peridium dingy grey; peridioles almost black at maturity ... *N. emodensis*
3. Exterior of peridium white to yellowish or buff; peridioles reddish brown to grey, never black 4
4. Peridioles 0.5–1.0 mm diam.; peridium 2–3-layered ..... *N. niveotomentosa*
4. Peridioles 0.9–1.3 mm diam.; peridium 6-layered ..... *N. shingbaensis*

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Geosmithia tibetensis sp. nov. and new *Gibellulopsis* and *Scopulariopsis* records from Qinghai-Tibet

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Abstract — A new *Geosmithia* species and new records of *Gibellulopsis nigrescens* and *Scopulariopsis canadensis* are described and illustrated from the Qinghai-Tibet Plateau Area, China. Specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP). Duplicates are kept in the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

Key words — dematiaceous hyphomycetes, soil fungi, taxonomy

Introduction

During a survey of soil dematiaceous hyphomycetes in China, several unusual species with phialidic conidia were isolated. One represents a new species and the others new records for China.

We describe as new *Geosmithia tibetensis,* which is illustrated from cultures grown on Czapek yeast extract agar (CYA; Pitt & Hocking 1985). *Geosmithia,* established by Pitt (1979), is characterized by asexual penicillate fruiting structures, typically with entirely rough-walled elements. The conidiogenous cells are cylindrical phialides. Conidia, which form in long chains, are smooth, ellipsoidal or subspherical, 0-septate, and hyaline to pale brown. Index Fungorum (2012) listed 19 taxa, and while Seifert et al. (2011) estimated that the genus might contain 18 valid species.

Gibellulopsis, established by Batista & Maia (1959), is characterized by subulate phialides and conidia that are hyaline and ellipsoidal to short-cylindrical. Chlamydospores are formed abundantly. Seifert et al. (2011) stated that the genus contains only one valid species. *Scopulariopsis,* established by

Bainier (1907), is characterized by penicillate conidiophores bearing cylindrical annellides, and conidia are elongate, ovate, or mitriform, hyaline to pale buff in mass. Although Index Fungorum (2012) listed 102 taxa (many infraspecific), Seifert et al. (2011) estimated that the genus may contain only 25 valid species.

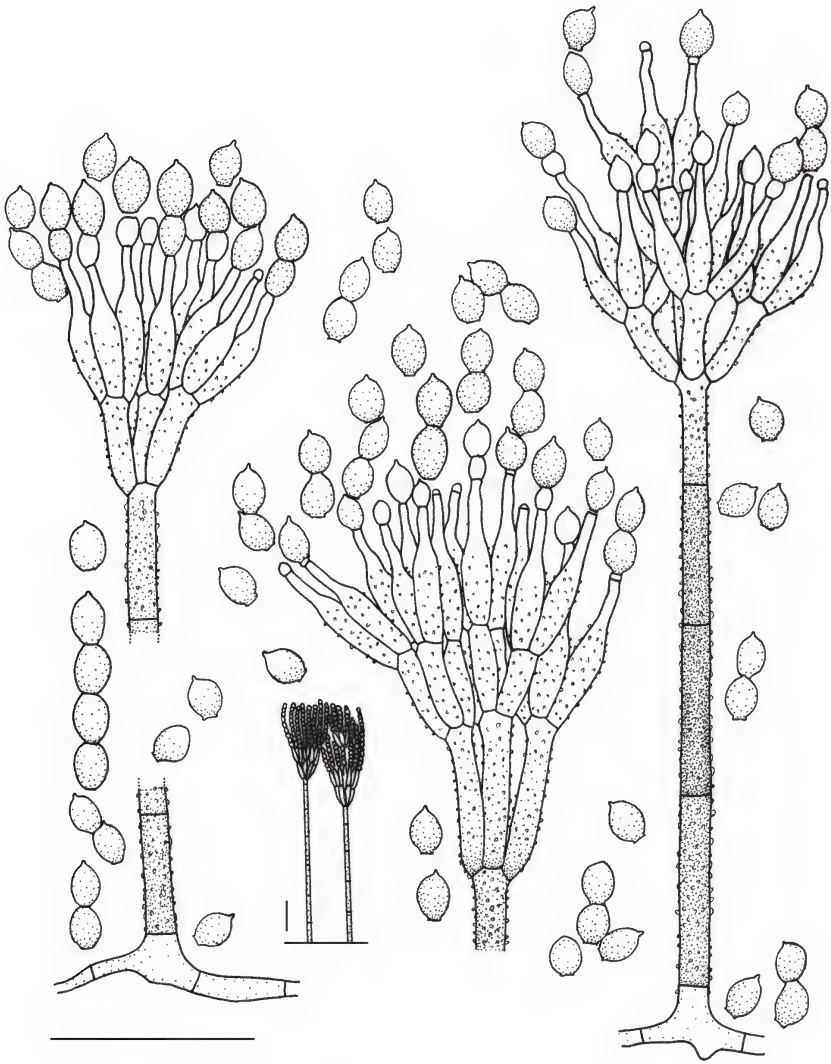

FIG. 1. *Geosmithia tibetensis* (ex holotype HSAUP II $_{07}$2000).
Conidia, conidiophores, and conidiogenous cells. Scale bars = 25 μm.

Geosmithia tibetensis Y.M. Wu & T.Y. Zhang, **sp. nov.** FIG. 1

MycoBank MB 802833

Differs from *Geosmithia fassatiae* by its larger conidia and phialidic conidiogenous cells without a distinct collar around the apical pore and from *G. lavendula* by its larger and paler brown conidia and moderately brown conidiophores.

TYPE: China, Tibet: Jiangzi, from a grassland soil, altitude 4100 m, 11 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$2000; **isotype** HMAS 196275).

ETYMOLOGY: in reference to the type locality.

COLONIES on CYA effuse, velvety, pale yellow-brown, Mycelium superficial or immersed, hyphae branched, septate, smooth, subhyaline to light brown, 2–3 μm wide. CONIDIOPHORES light brown, hyaline towards the apex, macronematous, mononematous, solitary, erect, septate, conspicuously to finely roughened, 60–80 μm long, 3–4 μm wide, penicillate, terminally bearing a small number of elements, typically monoverticillate to biverticillate, verrucose, 6–15 × 3–5 μm. CONIDIOGENOUS CELLS phialides, terminal, verrucose, hyaline, cylindrical, clavate or obclavate, 8–20 × 3–5 μm. CONIDIA smooth, ellipsoidal to oblong, pale brown, often distinctly pointed apex, with a subtruncate base, adhering in chains, 0-septate, 4.5–6 × 3–4.2 μm.

COMMENTS – Morphologically, *G. tibetensis* resembles *G. fassatiae* M. Kolařík et al. (Kolařík et al. 2005) and *G. lavendula* (Raper & Fennell) Pitt (Pitt 1979), but *G. fassatiae* has smaller conidia (3.5–4.5 × 2.5–3 μm) and conidiogenous cells often with a distinct collar around the apical pore, while *G. lavendula* has smaller, hyaline conidia (4.0–5.5 × 2.0–2.5 μm).

Gibellulopsis nigrescens (Pethybr.) Zare, W. Gams & Summerb.,
Nova Hedwigia 85: 477 (2007) FIG. 2

COLONIES on PDA effuse, dark grey, velvety. Mycelium superficial or immersed, hyphae branched, septate, smooth, hyaline to pale brown, 2–3 μm wide. CONIDIOPHORES smooth, pale brown, hyaline towards the apex, macronematous, mononematous, solitary or in groups, erect, septate, 180–230 μm long, 2.5–3.5 μm wide. CONIDIOGENOUS CELLS phialidic, smooth, hyaline, clavate or subulate, 20–45 × 1.5–2.5 μm. CONIDIA smooth, ellipsoidal to ovate, hyaline, often in chains, 0-septate, 4–6 × 1.5–2.5 μm. Chlamydospores abundant.

SPECIMEN EXAMINED: **CHINA. TIBET**: Xigaze, from a forest soil, altitude 3100 m, 11 Sept. 2007, Y.M. Wu (HSAUP II $_{07}$1082, HMAS 196276).

Gibellulopsis nigrescens is reported for the first time from China. Compared with the morphological characters of the species as described by Zare et al. (2007), both have hyaline conidia that are smooth, ellipsoidal to short-cylindrical, 0-septate, often in chains, and measure 4–6 × 1.5–2.5 μm and both form abundant

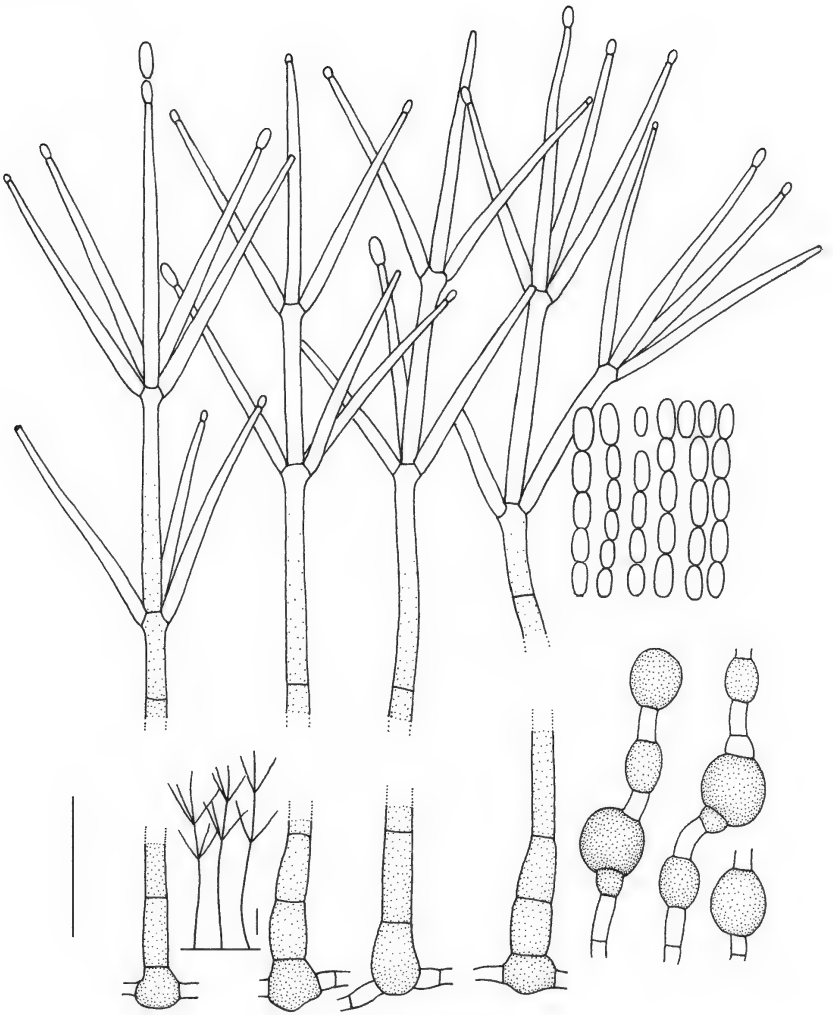

FIG. 2. *Gibellulopsis nigrescens* (HSAUP $II_{07}1082$).
Conidia, chlamydospores, conidiophores, and conidiogenous cells. Scale bars = 25 µm.

chlamydospores. We believe they are the same species. This is the only species known in the genus (Seifert et al. 2011).

Scopulariopsis canadensis F. J. Morton & G. Sm., Mycol. Pap. 86: 55 (1963) FIG. 3

COLONIES on MA (malt agar) effuse, fuscous-black, velvety. Mycelium superficial or partly immersed, hyphae branched, septate, smooth, hyaline

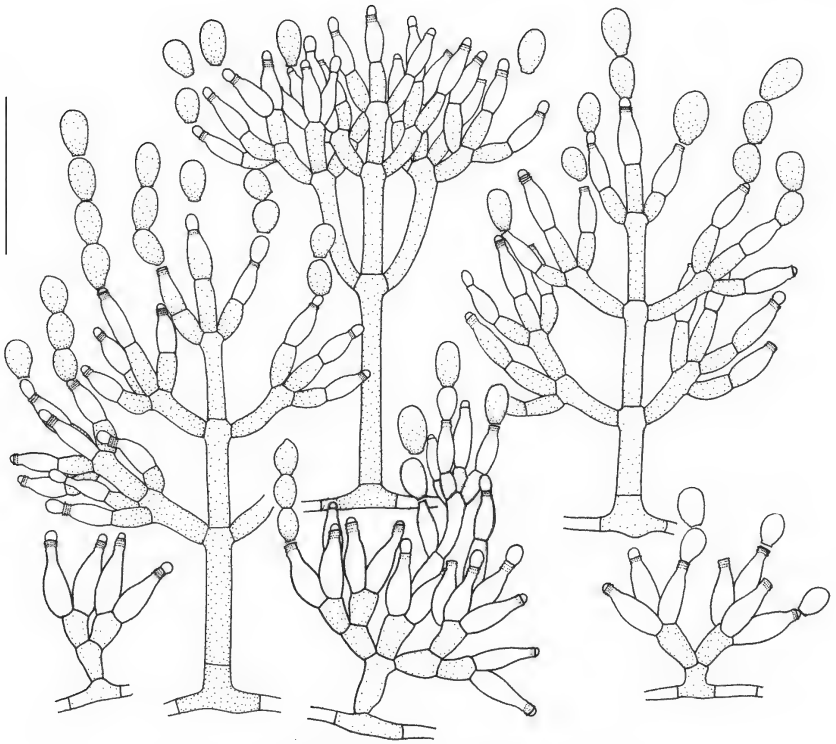

FIG. 3. *Scopulariopsis canadensis* (HSAUP II $_{07}$1481).
Conidia, conidiophores, and conidiogenous cells. Scale bar = 25 μm.

to pale brown, 1.5–2.5 μm wide. CONIDIOPHORES bearing annellophores in groups of 2–5 without metulae, or irregularly branched, pale brown, septate, smooth, 10–30 × 2.5–3.5 μm. CONIDIOGENOUS CELLS phialidic, terminal, smooth, hyaline, clavate or subulate, 8–15 × 3–6 μm. CONIDIA smooth, pale brown, ovate to oblong, with distinctly truncate base and rounded apex, often in chains, 0-septate, 6–8.5 × 4–5.5 μm.

SPECIMEN EXAMINED: **CHINA. TIBET**: Xigaze, from a grassland soil, altitude 4550 m, 20 Sept. 2007, Y.M. Wu (HSAUP II $_{07}$1481, HMAS 196277).

Scopulariopsis canadensis is reported for the first time from China. Compared with the descriptions by Morton & Smith (1963), both our specimens and the type have annellophores and pale brown conidia that are smooth, ovate to oblong, with distinctly truncate bases and rounded apices, often in chains, 0-septate, 6–8.5 × 4–5.5 μm. We believe they are the same species. In conidial shape, this species is most similar to *S. candida* (Vuillemin 1911), which differs in producing smaller (5–8 × 4–7 μm), and very pale conidia.

Acknowledgments

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New records of crustose lichens from China — 1

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Abstract — During recent studies on the crustose lichens from northeast China, *Cyphelium lucidum*, *Varicellaria lactea*, and *Diploschistes euganeus* were revealed as new records for China or mainland China. Illustrations and brief taxonomic descriptions are provided for these three newly reported species.

Key words — lichenized fungi, taxonomy, Greater Khingan Mountains

Introduction

In the course of our investigations on the lichen flora of northeast China, three new lichen records for China or mainland China were discovered. There are two mountain ranges in northeast China: the Greater Khingan Mountains and the Lesser Khingan Mountains. The average precipitation exceeds 500 millimeters annually in the mountains, and the northern half of the mountains is the coldest part of eastern China, with an extremely severe winter (mean temperature −28°C). The mountains are covered by forests of larch, birch, aspen, and pine. Specimens collected from other parts of China were also studied. Illustrations and brief taxonomic descriptions are provided for the newly reported taxa.

Materials & methods

All specimens examined are deposited in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU).

The lichen specimens were examined using an Olympus SZ 51 stereomicroscope and hand-cut sections under an Olympus CX 21 compound microscope. Photographs of the thallus were taken with an Olympus SZX16 camera with DP72. Color reactions (spot tests) were made using standard methods (Orange et al. 2001). Secondary metabolites of all specimens were identified using thin layer chromatography (TLC) as described by Elix et al. (1987) and Orange et al. (2001).

The new records

Cyphelium lucidum (Th. Fr.) Th. Fr., Gen. Heterolich. Eur.: 101 (1861) PLATE 1A

This species is characterized by a brilliant greenish yellow, areolate thallus; prominent, well dispersed, cup-like, black apothecia with sometimes lightly yellow pruinose discs; always yellow pruinose rim of the apothecia; ascospores 2-celled, about 10–20 × 7.5–10 μm, with a very rough surface; and the production of vulpinic acid. *Cyphelium notarisii* (Tul.) Blomb. & Forssell is another species with yellow-green thallus reported in China (Wu et al. 1997), but *C. notarisii* has immersed, epruinose apothecia and submuriform ascospores.

SPECIMEN EXAMINED — **CHINA. HEILONGJIANG PROV.**: TAHE COUNTY, Mengkeshan Forestry Centre, alt. 555 m, on bark of *Larix* sp., 14 Aug. 2009, Qiang Ren 2009197 (SDNU).

Varicellaria lactea (L.) Schmitt & Lumbsch, Mycokeys 4: 31 (2012) PLATE 1B

Diagnostic characters are the white-grey thallus with a regularly rimose-areolate surface; the round white soralia; the production of lecanoric acid; and the substrate of dry, siliceous or slightly calcareous rock. Apothecia were not seen in Chinese materials. The species might be confused with *Pertusaria hemisphaerica* (Flörke) Erichsen and *P. excludens* Nyl., but *P. hemisphaerica* has a pale blue-grey thallus with paler markedly convex soralia, which is C+ red,

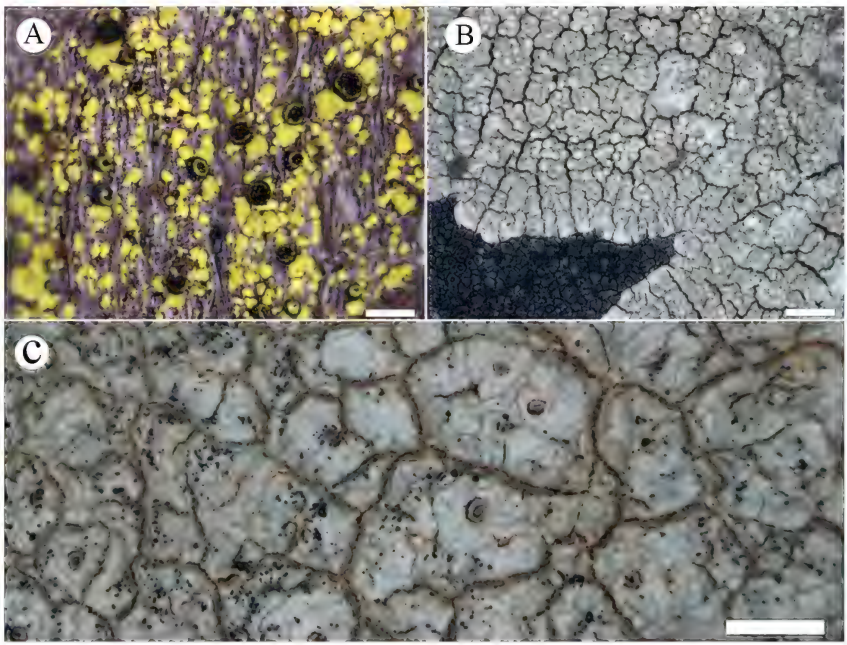

PLATE 1. A, *Cyphelium lucidum* (Qiang Ren 2009197); B, *Varicellaria lactea* (Qiang Ren 2012201); C, *Diploschistes euganeus* (Daifeng Jiang 20120613). Scale bars: A = 0.5 mm; B = 2 mm; C = 1 mm.

and occurs on trees. *Pertusaria excludens* also occurs on rocks but produces a C– medulla and soralia.

Specimens examined — **CHINA. Inner Mongolia**: Eerguna City, Eerguna Nature Reserve, 51°50.763′N 120°38.860′E, alt. 532 m, on siliceous rocks, 14 Aug. 2012, Qiang Ren 2012165 (SDNU); Moerdaoga Forest Park, 51°27.415′N 120°45.699′E, alt. 750 m, on siliceous rock, 10 Aug. 2012, Qiang Ren 2012060 (SDNU); Moerdaoga Forestry Bureau, Mt. Jiageda, 51°22.698′N 120°49.745′E, alt. 1255 m, on calcareous rock, 15 Aug. 2012, Qiang Ren 2012201 (SDNU). **Jinlin Prov.**: Yanji City, Wangqing County, Tianqiaoling Town, Mt. Tulaopo, alt. 1011 m, on siliceous rock, 1 Dec. 2007, Chao Yuan & Liyan Sun 20073409, 20073419 (SDNU). **Xizang**: Baxiu County, Ranwu Town, Laigu Glacier, alt. 4200 m, on siliceous rock,,32 Oct. 2007, Guoying Han 20072967 (SDNU).

Diploschistes euganeus (A. Massal.) J. Steiner, Verh. Zool.-Bot. Ges. Wien 69: 96 (1919) Plate 1c

This species is characterized by the thin whitish gray thallus that is rimose-areolate; perithecioid, immersed to semi-immersed ascomata; 8-spored asci with brown, broadly ellipsoid ascospores; absence of lichen metabolites; and a siliceous rock substrate. *Diploschistes euganeus,* which was reported from Taiwan (Aptroot 2011), is new to mainland China. *Diploschistes arabiensis* Lumbsch also occurs on siliceous rocks and lacks secondary metabolites but differs in its whitish thicker thallus, ridged apothecia, predominantly 6-spored asci, and distribution in Arabia and western North America.

Specimen examined — **CHINA. Liaoning**: Chaoyang City, Beipiao County, Mt. Dahei, alt. 1300 m, on siliceous rock, 14 Aug. 2011, Daifeng Jiang 20120613 (SDNU).

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The lichen genus *Graphis* from Vietnam

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Abstract — The status of *Graphis* (*Ostropales*: *Graphidaceae*) in Vietnam is presented, based on literature and observation of recently collected specimens. Eleven new records of the genus are described for the country. All important diagnostic characters of species are documented and supported by distribution, ecology, and illustrations. A key to the seventeen *Graphis* species in Vietnam is also provided.

Key words — evergreen forest, National Park, taxonomy, tropical

Introduction

The broad tropical continental biota experienced a long, undisturbed evolution that resulted into rich composition of old and mature forests (Chazdon & Whitmore 2002). According to general consensus the tropical old forests have a vast diversity of corticolous lichenized ascomycetes, particularly those of *Graphidaceae* (Aptroot & Sipman 1997; Sipman & Aptroot 2001; Feuerer & Hawksworth 2007; Archer 2009; Lücking et al. 2009, 2011; Rivas Plata et al. 2008, 2010, 2012). Vietnam, being placed in the tropical belt, contains dense continuous old forests exposed to a high and constant temperature regime and a prolonged humid climate. The Vietnamese northern and central high land ecosystems present an array of forest types, from deciduous to mixed deciduous and evergreen forest cover, mostly restored after the regions were declared National Parks (Jong et al. 2006). In Vietnam, there has not been much significant research on *Graphidaceae*. A more or less exhaustive account on Vietnam lichen mycota was published by Aptroot & Sparrius (2006) as a checklist, which accepts ca. 30 species of *Graphidaceae* (including thelotremoid taxa) for the country.

Graphis Adans., the largest graphidoid genus, comprises more than 300 species world-wide (Lücking et al. 2009). The genus, which has not thoroughly studied in Vietnam, is represented by only six species: *Graphis caesiella* Vain., *G. dussii* Vain., *G. leptogramma* Nyl., *G. librata* C. Knight, *G. proserpens* Vain., and *G. rimulosa* (Mont.) Trevis. In order to clarify the *Graphis* diversity in the country, a survey was conducted of three National Parks situated in northern and central highlands (Fig. 1a). About 400 specimens were collected from all three parks, including approximately 50 graphidaceous species representing *Acanthothecis*, *Diorygma*, *Fissurina*, *Graphis*, *Hemithecium*, *Platygramme*, *Sarcographa*, *Sarcographina*, and *Thecaria*. Our examination of the *Graphis* specimens has revealed eleven species new to Vietnam. Although the recorded *Graphis* species in no way encompass the full phytogeographical diversity of *Graphis* in Vietnam, they certainly indicate that many more species will be discovered in the country's tropical rain forests.

All species reported here share some common characteristics peculiar to *Graphis:* lirellate ascomata; carbonized proper exciple; non-amyloid (I–) hymenium; functionally unitunicate asci with apical wall thickening; simple, rather lax, apically granulate, ± conglutinate, 1–2 µm thick paraphyses; hyaline and amyloid (I+ blue), trans-septate to muriform ascospores with lens-shaped lumina; and *Trentepohlia* as photobiont (Archer 2009; Lücking et al. 2009). Ecologically, *Graphis* was reported mostly on the trees in secondary old growth forest and serves as a good indicator of forest health in National Parks in Vietnam.

The present account briefly describes the 11 *Graphis* species newly reported from Vietnam

Materials & methods

Chu Yang Sin, Yok Don, and Tam Dao National Parks of Vietnam were surveyed for their lichen diversity assessment in April 2012. The collected samples were preserved in lichen herbarium of Korean Lichen Research Institute, South Korea (KoLRI). The specimens were segregated up to generic level, and the members of *Graphis* were identified morphologically, anatomically and chemically. Morphological and anatomical characters of thallus and ascomata were observed under SMZ-168 (China) dissecting and Olympus BX50 (Tokyo, Japan) compound microscopes. The terminology for lirellae morphology follows Lücking et al. (2009). Lugol's solution (I) was used to check the amyloidity of ascospores. All measurements were taken on thin hand-cut sections of ascomata mounted in tap water. For color spot reaction tests and thin layer chromatography (TLC), methodology provided by Orange et al. (2010) was followed. For TLC, solvent system C (toluene 85%, acetic acid 15%) was used. The key characters of the species were determined by consulting recent literature on *Graphis* (Staiger 2002; Archer 2009; Lücking et al. 2009).

Taxonomy

Graphis caesiocarpa Redinger, Ark. Bot. 27A(3): 23, 1935. Pl. 1B

Thallus corticolous, epiperidermal, crustose, continuous, rough, whitish or creamy grey, 100–150 µm thick in cross section; cortex layer indistinct to absent; algal layer indistinct to 10 µm; medulla epi- to endoperidermal, densely studded with crystals, ≤140 µm; prothallus blackish.

Ascomata lirelliform, immersed; lirellae scattered, 5–10 mm long, black; labia entire, whitish to creamy pruinose; disc slit-like, concealed (*caesiella*-morph); proper exciple completely carbonized, convergent, ± round, 50–140 µm wide, basally sometimes merged with hypothallus; epihymenium indistinct to absent; hymenium clear, 100–120 µm high; hypothecium hyaline, indistinct to 20 µm high; ascus clavate, 8-spored; ascospores hyaline, fusiform, mostly transversely 15–20-loculate, 50–70(–80) × 10–11 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid detected in TLC.

Distribution & ecology: Neotropics and Eastern Palaeotropics (Lücking et al. 2009); in Vietnam, it was found growing at 700–800 m with other *Graphis* and *Diorygma* species on tree trunks at medium height.

Specimen examined: **VIETNAM. Dak Lak province:** Yok Don National Park, 12°51′20.0″N 107°45′58.1″E, alt. ca. 760 m, on tree, 22 April 2012, Hur & Oh VN120214–1 (KoLRI).

Remarks: *Graphis assimilis* Nyl. has a similar distribution and also contains norstictic acid but differs from *G. caesiocarpa* in short and sparsely branched lirellae (*lineola*-morph). The ascospore size of the examined material, which exceeds the range cited in Lücking et al. (2009), reaches that of *G. marginata* Raddi, which has entirely different lirellae morphology (*marginata*-morph). Pending additional collections, the material is provisionally placed in *G. caesiocarpa*.

Graphis cervinonigra Zahlbr., Feddes Repert. 31: 210, 1933. Pl. 1C

Thallus corticolous, epiperidermal, crustose, continuous, ± smooth, ± brownish green to grey-green, 100–150 µm thick in cross section; cortex layer 15–20 µm; algal layer continuous, 20–40 µm; medulla mostly endoperidermal, 80–100 µm, with inclusions of crystals; prothallus indistinct to blackish.

Ascomata lirelliform, prominent; lirellae aggregate, 5–7 mm long, black, ± stellately branched (*coarctata*-morph); labia entire, epruinose; disc slit-like, concealed; proper exciple completely carbonized, convergent, ± round, 65–75 µm wide; epihymenium indistinct to absent; hymenium inspersed, 100–120 µm high; hypothecium hyaline, indistinct to 20 µm high; ascus clavate, 8-spored; ascospores hyaline, fusiform, mostly transversely 9–10-loculate, 20–35 × 6–8 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid detected in TLC.

Distribution & ecology: Eastern Palaeotropics (Lücking et al. 2009); in Vietnam, *Graphis cervinonigra* was reported at 70–1000 m on tree bark in evergreen forest. The other lichens growing in surrounding environment were the members of *Diorygma*, *Letrouitia*, and *Ocellularia*.

Specimens examined: **VIETNAM. Vinh Phuc province:** Tam Dao National Park, Tay Thien Mt., 21°26′52.2″N 105°38′10.8″E, alt. ca. 936 m, on tree, 26 April 2012, Hur & Oh VN120372, VN120374 (KoLRI); 21°27′10.8″N 105°38′58.2″E, alt. ca. 77 m, on tree, 25 April 2012, Hur & Oh VN120349 (KoLRI).

Remarks: The species resembles *Graphis centrifuga* Räsänen in having branched lirellae, entire labia, completely carbonized proper exciple, transversely septate ascospores, norstictic acid in thallus, and a similar geographical distribution. *Graphis centrifuga* differs in long, radially branched lirellae and smaller ascospores. *Graphis gonimica* Zahlbr. shares similar anatomical and chemical characters but differs from *G. cervinonigra* in sparsely to irregularly branched lirellae (*lineola-* or *deserpens-* morph).

Graphis cycasicola A.W. Archer & Elix, Australas. Lichenol. 61: 19, 2007. Pl. 1d

Thallus corticolous, epiperidermal, crustose, continuous, smooth, ± bulging (due to bark texture), whitish-grey or green to grey-green, 170–200 µm thick in cross section; cortex layer distinct, 10–15 µm; algal layer continuous, 30–50 µm; medulla mostly endoperidermal, studded with crystals; prothallus indistinct to blackish.

Ascomata lirelliform, emergent; lirellae scattered, simple, straight to sinus, short to 5 mm long, branched, with thick, complete thalline margin (*subserpentina*-morph); labia entire; disc slit-like, concealed; proper exciple completely carbonized, convergent, ≤170 µm wide; epihymenium ≤15 µm, greyish, granular; hymenium clear, 120–150 µm high; hypothecium hyaline, indistinct to 20 µm high; ascus clavate, 1-spored; ascospores hyaline, ellipsoidal, muriform, multicelled, 80–110 × 25–40 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid detected in TLC.

Distribution & ecology: Eastern Palaeotropics (Lücking et al. 2009); in Vietnam, the species was growing luxuriantly at 700–800 m above chest height on thick rough tree trunks.

Specimen examined: **VIETNAM. Dak Lak province:** Yok Don National Park, 12°51′20.0″N 107°45′58.1″E, alt. ca. 760 m, on tree, 22 April 2012, Hur & Oh VN120216 (KoLRI).

Remarks: Both morphologically as well as chemically, *G. cycasicola* is similar to *G. subserpentina*, which is distinguished by a laterally carbonized proper exciple.

Graphis epiphloea Zahlbr., Feddes Repert. 31: 210, 1933. Pl. 1E

Thallus corticolous, epiperidermal, crustose, continuous, smooth, shiny, green to greyish-green, 100–300 µm thick in cross section; cortex layer 20–25 µm; algal layer continuous, 30–50 µm; medulla mostly endoperidermal; prothallus indistinct.

Ascomata lirelliform, ± immersed; lirellae aggregate, radially branched, ≤20 mm long, covered by thick complete thalline margin (*subserpentina*-morph); labia entire; disc slit-like, concealed; proper exciple apically carbonized, convergent, ≤60–70 µm wide; epihymenium ≤15 µm, greyish, granular; hymenium clear, 150–170 µm high; hypothecium hyaline, ≤40 µm high; ascus clavate, 6–8-spored; ascospores hyaline, fusiform, transversely septate, 15–20-loculate, 90–100(–140) × 18–20 µm.

Chemistry: K+ yellow, PD+ yellow-orange, C–; stictic acid detected in TLC.

Distribution & ecology: Eastern Palaeotropics (Lücking et al. 2009); in Vietnam, the species was collected at ca. 1000 m from a tree trunk above chest level.

Specimen examined: **VIETNAM. Vinh Phuc province:** Tam Dao National Park, 21°27′21.7″N 105°39′00.8″E, alt. ca. 1092 m, on tree, 25 April 2012, Hur & Oh VN120319 (KoLRI).

Remarks: *Graphis valparaiensis* Adaw. & Makhija also produces entire labia, apically carbonized proper exciple, clear hymenium, transversely septate large ascospores, and stictic acid but differs from *G. epiphloea* in sparsely branched lirellae with lateral thalline margin (*lineola*-morph). The examined material was rather poor, and further collection of the material is needed.

Graphis handelii Zahlbr., Symb. Sinic. 3: 44, 1930. Pl. 1F

Thallus corticolous, epiperidermal, crustose, continuous, ± smooth, green to grey-green, 100–150 µm thick in cross section; cortex layer 10–15 µm; algal layer continuous, 30–40 µm; medulla 80–100 µm to mostly endoperidermal, with inclusions of crystals; prothallus indistinct to blackish.

Ascomata lirelliform, prominent; lirellae aggregate, short to 5 mm long, black, unbranched; labia entire, epruinose; disc exposed, epruinose (*handelii*-morph); proper exciple laterally carbonized, convergent, 50–70 µm wide; epihymenium indistinct to absent; hymenium inspersed, ≤100 µm high; hypothecium indistinct; ascus clavate, 8-spored; ascospores hyaline, fusiform, transversely septate, 9–10-loculate, 20–30 × 6–7 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid detected in TLC.

Distribution & ecology: Pantropical (Lücking et al. 2009); in Vietnam, the species was collected below 100 m from smooth barked trees, where it was growing with members of the lecanoroid and lecidioid groups.

Specimens examined: **VIETNAM. Vinh Phuc province:** Tam Dao National Park, Tay Thien Mt., 21°27′53.3″N 105°35′06.6″E, alt. ca. 77 m, on tree, 26 April 2012, Hur & Oh VN120389, VN120390, VN120392 (KoLRI).

Remarks: *Graphis crebra* Vain. and *G. aperiens* Müll. Arg., which are most similar to *G. handelii*, have distinctly white pruinose ascomatal discs (*scripta*-morph). Moreover, *G. aperiens* has a completely carbonized proper exciple. The examined material could be confused with *G. plumierae* Vain., which has pruinose labia with closed ascomatal discs (*caesiella*-morph) and a different geographical distribution.

Graphis japonica (Müll. Arg.) A.W. Archer & Lücking, Lichenologist 41: 437, 2009. Pl. 1G

Thallus corticolous, epiperidermal, crustose, continuous, bulging (due to bark texture), smooth to ± verrucose, shiny, grey-green, pale-green or yellowish green , ≤170 µm thick in cross section; cortex layer 20–30 µm; algal layer continuous, 50–60 µm; medulla epi to endoperidermal, ≤100 µm; prothallus indistinct to blackish.

Ascomata lirelliform, ± immersed; lirellae intensely branched, branching leads the length of lirellae ≤30 mm, covered by thick complete and bulging thalline margin (*subserpentina*-morph); labia entire; disc slit-like, concealed; proper exciple apically to laterally carbonized, convergent, ≤20–70 µm wide; epihymenium ≤10 µm, greyish, granular; hymenium clear, 150–170 µm high; hypothecium hyaline, 30–40 µm high; ascus clavate, 2–8-spored; ascospores hyaline, ellipsoidal, muriform, 10–15 × 4–6 loculate, 70–80 × 18–22 µm.

Chemistry: K+ yellow, PD+ yellow-orange, C–; stictic acid detected in TLC.

Distribution & ecology: Eastern Palaeotropics (Lücking et al. 2009); in Vietnam, the species was collected at 600–800 m, where it was largely spread over thick tree trunks in an evergreen forest, usually below chest level.

Specimens examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′04.4″N 108°20′39.0″E, alt. ca. 622 m, on tree, 20 April 2012, Hur & Oh VN120062 (KoLRI); 12°27′57.0″N 108°20′34.9″E, alt. ca. 780 m, on tree, 21 April 2012, Hur & Oh VN120139 (KoLRI).

Remarks: *Graphis japonica* is a distinct species with pale-green to grey-green thallus and a very long branched lirellae, which sometimes appear as grooves on tree bark. The similar *G. streblocarpa* (Bél.) Nyl. differs in 1–2-spored asci and a Neotropical distribution (Lücking et al. 2009).

Graphis renschiana (Müll. Arg.) Stizenb., Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1889–90: 184. 1891. Pl. 1H

Thallus corticolous, epiperidermal, crustose, continuous, ± smooth, green to grey-green, ≤100 µm thick in cross section; cortex layer indistinct to 10 µm;

algal layer continuous, 20–30 µm; medulla 30 µm to mostly endoperidermal, with inclusions of crystals; prothallus indistinct.

Ascomata lirelliform, immersed to erumpent; lirellae radially to irregularly branched (*deserpens*-morph), 10–15 mm long, black; labia entire; disc concealed; proper exciple laterally carbonized, convergent, 40–50 µm wide; epihymenium indistinct to absent; hymenium clear, ≤100 µm high; hypothecium 20–25 µm high; ascus clavate, 2–8-spored; ascospores hyaline, ellipsoidal, muriform, 7–10 × 5–6-loculate, 18–25 × 6–8 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid detected in TLC.

Distribution & ecology: Pantropical (Lücking et al. 2009); in Vietnam, the species was collected at 700–800 m, where it was growing luxuriantly with other graphidoid taxa above chest level.

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°27′50.0″N 108°20′34.9″E, alt. ca. 780 m, on tree, 21 April 2012, Hur & Oh 120141 (KoLRI).

Remarks: The over mature ascomata of *G. renschiana* are slightly striate as in *G. neoelongata* Lücking, which also contains norstictic acid but differs in also producing stictic acid and its occurrence limited (thus far) to the Neotropics (Lücking et al. 2009). The other similar species, *G. nadurina* Aptroot, differs in prominent, unbranched, very short lirellae with apically thick, complete thalline margin (*cleistomma*-morph). The lirellae of the observed specimen do not fully agree with the description in *G. renschiana* and so additional collections are needed to clarify the identification.

Graphis schroederi Zahlbr., Ann. Mycol. 19: 228, 1921. Pl. 11

Thallus corticolous, epiperidermal, crustose, continuous, ± smooth, green to grey-green, 200–250 µm thick in cross section; cortex layer distinct, ≤20 µm; algal layer continuous, 30–50 µm; medulla mostly endoperidermal, 140–180 µm, with inclusions of crystals; prothallus indistinct to blackish.

Ascomata lirelliform, prominent; lirellae aggregate, radially branched, apically covered by thick thalline margin (*subserpentina*-morph), 5–10 mm long, black; labia entire; disc concealed; proper exciple completely carbonized, convergent, 30–60 µm wide, basally merged with hypothallus; epihymenium indistinct to absent; hymenium clear, ≤200 µm high; hypothecium indistinct; ascus clavate, 8-spored; ascospores hyaline, fusiform, transversely septate, 14–23-loculate, 70–90(–130) × 14–17 µm.

Chemistry: K+ yellow, PD+ yellow-orange, C–; stictic acid detected in TLC.

Distribution & ecology: Palaeotropics (Lücking et al. 2009); in Vietnam, the species was collected at 900–1000 m from smooth barked trees.

Specimen examined: **VIETNAM. Vinh Phuc province:** Tam Dao National Park, 21°27′13.1″N 105°38′51.2″E, alt. ca. 990 m, on tree, 25 April 2012, Hur & Oh VN120310 (KoLRI).

Remarks: The sympatric *G. subassimilis* Müll. Arg. also has entire labia, completely carbonized proper exciple, clear hymenium, transversely septate ascospores, and stictic acid but differs from *G. schroederi* in comparatively smaller ascospores, lateral thalline margin, and pruinose labia (*caesiella*-morph). The other similar species, *G. rustica* Kremp., differs from *G. schroederi* in elongate and irregularly branched lirellae with thick lateral thalline margins (*marginata*-morph).

Graphis subserpentina Nyl., Acta Soc. Sci. Fenn. 7: 465, 1863. Pl. 1J

Thallus corticolous, epiperidermal, crustose, continuous, smooth, crystalline, whitish-green to creamy green or off-white, ≤150 µm thick in cross section; cortex layer indistinct; algal layer continuous, 100–130 µm; medulla mostly endoperidermal, studded with crystals; prothallus blackish.

Ascomata lirelliform, ± erumpent; lirellae aggregate, irregularly branched, 5 to 7 mm, covered by lateral thalline margin (*subserpentina*-morph); labia entire; disc slit-like, concealed; proper exciple laterally carbonized, convergent, ≤230 µm wide in mature ascomata; epihymenium indistinct; hymenium clear, 125–150 µm high; hypothecium hyaline, ≤40 µm high; ascus clavate, 1-spored; ascospores hyaline, ellipsoidal, muriform, multicelled, 80–100 × 20–25 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; trace of norstictic acid detected in TLC.

Distribution & ecology: Palaeotropics (Lücking et al. 2009); in Vietnam, the species was recorded from tree bark at 300–400 m.

Specimen examined: **VIETNAM. Dak Lak province:** Yok Don National Park, 12°51′20.0″N 107°45′58.1″E, alt. ca. 760 m, on tree, 22 April 2012, Hur & Oh VN120215 (KoLRI).

Remarks: *Graphis subserpentina* morphologically resembles *G. streblocarpa*, which differs in containing stictic acid. The chemically similar *G. insulana* (Müll. Arg.) Lücking & Sipman has an inspersed hymenium.

Graphis subvittata Adaw. & Makhija, Mycotaxon 99: 322, 2007. Pl. 1K

Thallus corticolous, epiperidermal, crustose, continuous, ± smooth (appears uneven due to bark texture), grey-green to greyish, 140–200 µm thick in cross section; cortex ≤20 µm; algal layer continuous, 20–30 µm; medulla 100–150 µm, with inclusions of crystals; prothallus blackish.

Ascomata lirelliform, erumpent to prominent; lirellae mostly scattered to aggregate, irregularly branched, with basal to lacking thalline margin (*striatula*-morph), ≤10 mm long, black; labia striate; disc concealed; proper exciple peripherally carbonized, convergent, 40–80 µm wide; epihymenium indistinct

to absent; hymenium clear, ≤100 µm high; hypothecium distinct, 20–25 µm high; ascus clavate, 8-spored; ascospores hyaline, fusiform, transversely septate, 9–10-loculate, 20–30 × 6–7 µm.

Chemistry: K+ yellow turning red, PD+ yellow-orange, C–; norstictic acid and traces of stictic acid detected in TLC.

Distribution & ecology: Eastern Palaeotropics (Lücking et al. 2009); in Vietnam, the species was collected from rough bark of trees, where it was spreading luxuriantly at an altitude between 700–800 m.

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°27′50.0″N 108°20′34.9″E, alt. ca. 780 m, on tree, 21 April 2012, Hur & Oh VN120184 (KoLRI).

Remarks: *Graphis subvittata* is most similar in thallus compound and lirellae morphology to *G. nigroglauca* Leight., which differs in larger ascospores and lacking a thalline margin. The examined specimen is comparable to *G. schiffneri* Zahlbr. in striated labia and the presence of norstictic acid, but *G. schiffneri* has a completely carbonized exciple and *tenella*-morph lirellae and lacks stictic acid.

Graphis supracola A.W. Archer, Austral. Syst. Bot. 14: 267, 2001. Pl. 1L

Thallus corticolous, epiperidermal, crustose, continuous, smooth, greyish-green, thin, 70–100 µm thick in cross section; cortex layer indistinct to absent; algal layer continuous, 40–60 µm; medulla mostly endoperidermal, indistinct, studded with crystals; prothallus indistinct to blackish.

Ascomata lirelliform, immersed; lirellae scattered, radially branched, 5 to 10 mm long, with thin lateral thalline margin; labia entire, thinly white pruinose (*caesiella*-morph); disc slit-like, concealed; proper exciple completely carbonized, convergent, ≤30–80 µm wide; epihymenium ≤15 µm, brownish, granular; hymenium clear, ≤100 µm high; hypothecium hyaline, ≤20 µm high; ascus clavate, 8-spored; ascospores hyaline, ellipsoidal, transversely septate, 20–30 × 7–8 µm.

Chemistry: K–, PD+ yellow-orange, C–; protocetraric acid detected in TLC.

Distribution & ecology: Pantropical (Lücking et al. 2009); in Vietnam, the species was recorded near a waterfall, where it was growing in small patches with *Letrouitia leprolytoides* S.Y. Kondr. & Elix and other crustose species on thick rough tree bark at 300–400 m.

Specimen examined: **VIETNAM. Dak Nong province:** Dray Nur Waterfall, 12°33′06.3″N 107°73′19.6″E, alt. ca. 371 m, on tree, 19 April 2012, Hur & Oh VN120008 (KoLRI).

Remarks: The examined material of *G. supracola* was rather small, but identifiable. It agrees well with all diagnostic characters except thallus color,

which Archer (2009) described as pale-fawn. The similar *G. distincta* Makhija & Adaw. differs in exposed pruinose ascomatal discs and containing stictic and constictic acids as an additional compounds.

Key to *Graphis* species recorded from Vietnam

The six previous records and eleven new records from Vietnam are summarized in this key. Diagnostic characters of the earlier recorded species are from Lücking et al. (2009).

1. Labia striate 2
1. Labia entire 4
2. Proper exciple completely carbonized; ascospores 30–80 µm long ... ***G. rimulosa***
2. Proper exciple apically to peripherally carbonized 3
3. Norstictic and stictic acids present; ascospores 20–30 µm long ..... ***G. subvittata***
3. No chemical substances; ascospores 20–50 µm long ..... ***G. proserpens***
4. Proper exciple completely carbonized 5
4. Proper exciple apically to laterally carbonized 8
5. Hymenium inspersed; lirellae long and radially branched; ascospores 20–35 × 6–8 µm ..... ***G. cervinonigra***
5. Hymenium clear 6
6. Ascospores muriform, 80–110 × 25–40 µm; norstictic acid present. ***G. cycasicola***
6. Ascospores transversely septate; chemistry variable 7
7. Norstictic acid present; ascospores 50–70 × 10–11 µm ..... ***G. caesiocarpa***
7. Stictic acid present; ascospores 70–90 × 14–17 µm ..... ***G. schroederi***
8. Proper exciple apically to laterally carbonized; stictic acid present 9
8. Proper exciple laterally carbonized, chemistry variable 10
9. Proper exciple apically or laterally carbonized; ascospores muriform, 70–80 × 18–22 µm ..... ***G. japonica***
9. Proper exciple apically carbonized; ascospores transversely septate, 90–100 × 18–20 µm ..... ***G. epiphloea***
10. Hymenium inspersed; norstictic acid present; ascospores 20–30 × 6–7 µm ..... ***G. handelii***
10. Hymenium clear 11
11. Ascospores muriform; norstictic acid present 12
11. Ascospores transversely septate 13
12. Asci 1-spored; ascospores 80–100 × 20–25 µm ..... ***G. subserpentina***
12. Asci 2–8-spored; ascospores 18–25 × 6–8 µm ..... ***G. renschiana***
13. Protocetraric acid; ascospores 20–30 × 7–8 µm ..... ***G. supracola***
13. Stictic and hypostictic, or norstictic acids present 14
14. Stictic and hypostictic acids present; lirellae narrow, with gently sloping lateral thalline margin; ascospores 44–65 µm long ..... ***G. leptogramma***
14. Norstictic acid present 15

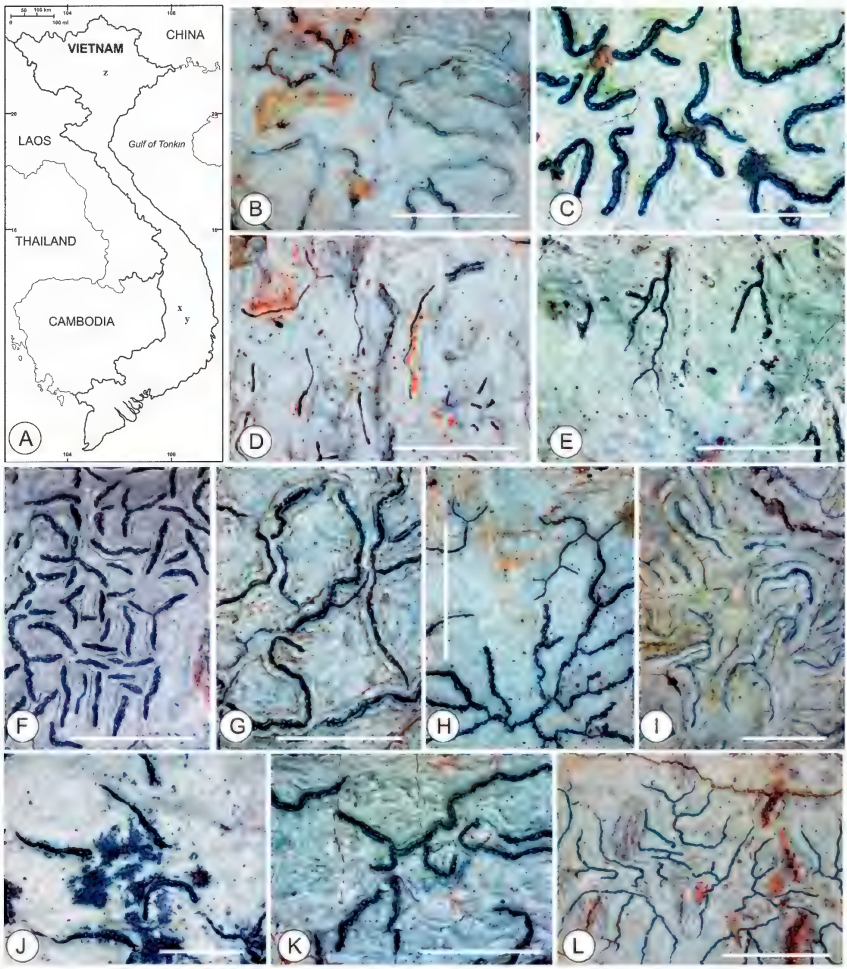

Plate 1. A. Map of Vietnam showing collection sites in northern and central highlands (x = Chu Yang Sin NP; y = Yok Don NP; z = Tam Dao NP); B. *Graphis caesiocarpa;* C. *G. cervinonigra;* D. *G. cycasicola;* E. *G. epiphloea;* F. *G. handelii;* G. *G. japonica;* H. *G. renschiana;* I. *G. schroederi;* J. *G. subserpentina;* K. *G. subvittata;* L. *G. supracola.* Scale bars: B–D, F–L = 5 mm; E, G= 10 mm.

15. Lirellae prominent to sessile, with thick lateral thalline margin, very short and unbranched; corticolous..... ***G. dussii***
15. Lirellae immersed to erumpent; corticolous and saxicolous 16
16. Lirellae elongate and irregularly branched; labia distinctly white pruinose.....***G. caesiella***
16. Lirellae short and sparsely branched; labia epruinose..... ***G. librata***

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Setosynnema yunnanense sp. nov. from submerged decaying leaves

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Abstract — *Setosynnema yunnanense,* a new species isolated from decaying leaves collected in Xishuangbanna, Yunnan Province, China, is described. It is placed in *Setosynnema* based on its chain-like conidia constricted at some septa (resulting in adjacent cells being connected by a narrow isthmus). It differs from the type species, *S. isthmosporum,* by its deeper and more frequent constrictions and its shorter conidia.

Key Words — tubercularіaceous fungus, systematic, aquatic fungi

Introduction

Setosynnema D.E. Shaw & B. Sutton was erected to accommodate a single anamorphic fungal species, *S. isthmosporum* D.E. Shaw & B. Sutton from Papua New Guinea and Australia, characterized by scattered brown synnematous conidiomata with whorls of setae near the apex (Shaw & Sutton 1985). The conidia are holoblastic, filiform, widest at the centre, and with an isthmus at the central septum tapered towards each end. No additional species have been described. Subsequently, Nawawi (1985) isolated this species from Malaysia, Matsushima (1996) from Japan, and Marvanová & Hywel-Jones (2000) from Thailand. According to K. Seifert (pers. comm.), *Synnematophora* K.R. Sridhar & Kaver. (represented by a single species, *S. constricta* K.R. Sridhar & Kaver. from India; Sridhar & Kaveriappa 2002) is a synonym of *Setosynnema*.

China has an enormous diversity of mycota occurring on dead branches and rotten submerged wood and leaves, and many anamorphic fungi collected

[#] Ya-Li Bai & Jian-Ying Li contributed equally to this work

in southern China have recently been published (Ma et al. 2010, 2011a,b, 2012a,b; Ren et al. 2011, 2012; Zhang et al. 2009a,b, 2011, 2012). During our survey of fungi growing on rotten submerged leaves, we isolated an unknown *Setosynnema* species, which we describe and illustrate here as a new species. We also isolated *S. isthmosporum* from Yunnan Province, a new record for China.

Materials & methods

The culture was isolated from dicotyledonous leaves submerged in a river in Yunnan. A 2–4 × 4–5 cm rotten leaf was spread on the surface of CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) for ten days; single conidia were isolated using a sterilized toothpick while viewing with a CX31 microscope and cultivated on CMA in Petri plates. Morphological observations were made from CMA after incubation at 28°C for one week; pure cultures and a permanent slide were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF, formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan).

Taxonomy

Setosynnema yunnanense Y.L. Bai & Z.F. Yu, **sp. nov.** PLATE 1

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Differs from *Setosynnema isthmosporum* by its shorter conidia that have fewer septa and more than one septum constricted to form an intercellular isthmus.

TYPE: PR China, Yunnan Province, Xishuangbanna city, Tropical Botanical Garden, Chinese Academy of Science, 21°55′N 101°16′E, elev. 567 m, in a river on submerged leaves of an unidentified dicotyledonous plant, July 2012, Y.L. Bai (**Holotype** YMF1.03964 [dried agar culture]; permanent slide, YMF1.039641; ex-type cultures, YMF1.039642).

ETYMOLOGY: *yunnanense* refers to the province in which the species was found.

Colonies effuse, brown to dark brown, attaining 35 mm diam. after 7 days on CMA at 28°C. Mycelium partly superficial, partly immersed in substratum, composed of branched, septate, tuberculate hyphae. Vegetative hyphae 2.5–3.5 µm wide, pale brown to brown. Conidiomata are usually massive, stout, synnematous, solitary or in aggregates of 1–3, brown, made up of parallel closely packed brown hyphae, with an expanded spore producing head. Setae absent in pure culture but present on natural substrate. Conidiophores arising close to one another, and flock together closely, septate, smooth, subhyaline to pale brown towards base, hyaline towards apex, sometimes 1–3 irregularly branched, with primary, secondary and tertiary branches, 77–160 µm tall, 3.0–5.0 µm wide, apex somewhat truncate to rounded, invariably with two scars marking position of branches or conidiogenous cells attachment. Conidiogenous cells terminal, apices somewhat rounded or truncate, with two slightly protuberant scars, clavate to cylindrical, hyaline, integrated. Conidia produced in a whitish mass emerging from apex of synnemata, hyaline, sometimes becoming pale

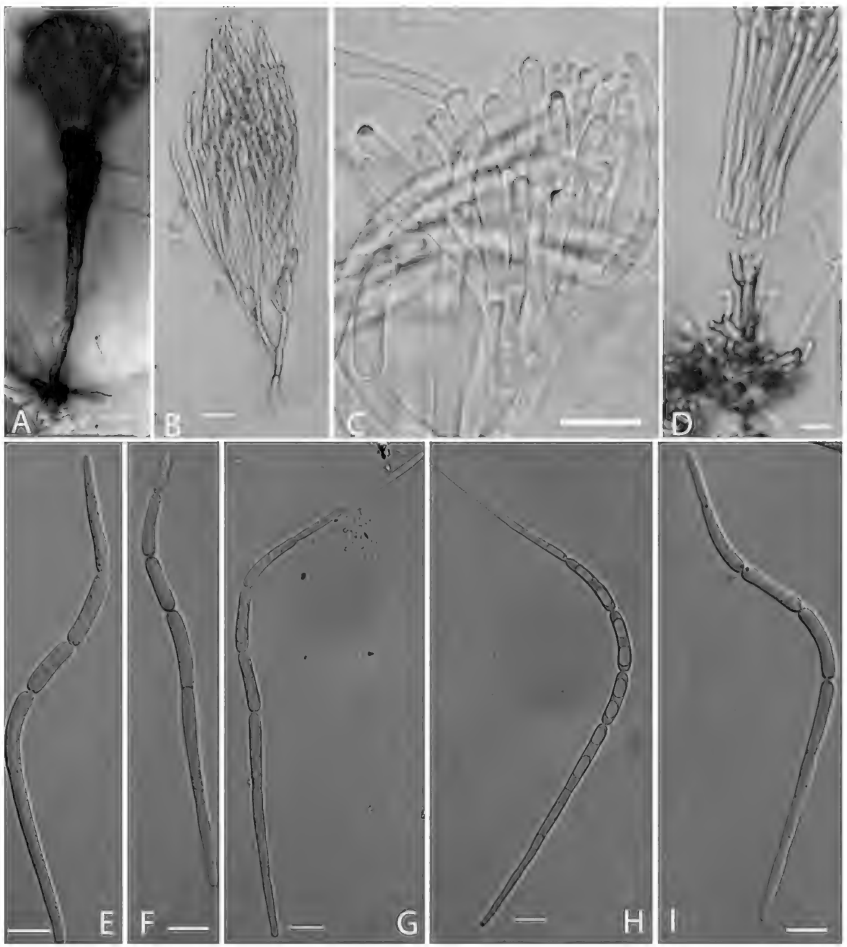

Plate 1. *Setosynnema yunnanense* (holotype YMF1.03964, from pure culture). A. Conidiomata. B. Branched conidiophores bearing conidia in clusters. C. Conidiogenous cells. D. Deciduous conidia and conidiogenous cells. E–I. Conidia. Scale bars: A = 50 μm; B = 20 μm; C–I = 10 μm.

whitish brown, acrogenous, filiform, curved, 94–154 μm long, 2.0–3.0 μm wide at the widest part near the centre, tapering to 1.0–1.5 μm at each end, but distal end often narrower. Uniseriate chains of 6–8 cells, with 2–4 (mainly 3) of the septa between the middle cells constricted to form a narrow isthmus. The number and position of the constricted septa within the conidium is variable; there are 3(–4) constricted septa near the middle of each conidium, with 0–2 unconstricted septa at the distal end and 2 unconstricted septa at the proximal end.

Discussion

Setosynnema yunnanense fits well within *Setosynnema* based on its synnematous conidiomata and filiform septate conidia with strong constrictions at some septa that result in the central cells being connected by narrow isthmi.

However, the conidia of *S. yunnanense* differ from those of *S. isthmosporum*, which has longer conidia ((189–)200.5–260(–290) μm) that are 7–8-septate with only one septum constricted to an isthmus and with similarly wide proximal and distal portions.

We did not see any setae in the pure culture of *S. yunnanense* as noted for *S. isthmosporum*, although we did observe setae on the decaying leaves from which the type species was isolated. In *S. isthmosporum*, setae were rarely produced in pure culture (Shaw & Sutton 1985: 30). This variability in setal production supports the proposed synonymy of *Setosynnema* and *Synnematophora*, for which the presence/absence of setae was proposed as a major differentiating character (Marvanová 2011:876).

The conidiogenous cell apices vary in different strains of the type species. The type strain produces 1–3 applanate, denticulate, unthickened condiogenous loci (Shaw & Sutton 1985). In the Japanese strain, the denticles are not as obvious and are only slightly visible even under the SEM (Matsushima 1996), while two conidiogenous loci are easily observed in the strain from Malaysia (Nawawi 1985). The conidiogenous cell apices of *S. yunnanense* are similar in shape to those of the type strain of *S. isthmosporum*.

Acknowledgements

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Two earth-tongue genera new for Turkey

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Abstract — The genera *Spathulariopsis* (*Cudoniaceae*) and *Trichoglossum* (*Geoglossaceae*) are recorded from Turkey for the first time, based on the collections of *Spathulariopsis velutipes* and *Trichoglossum hirsutum*. Short descriptions and photographs of the taxa are provided.

Key words — *Ascomycota*, biodiversity, macrofungi

Introduction

Earth-tongues, including the genera *Geoglossum* Pers., *Leotia* Pers., *Microglossum* Gillet, *Spathularia* Pers., *Spathulariopsis* Maas Geest., and *Trichoglossum* Boud., are among the most widely distributed groups of fungi in the division *Ascomycota*. They produce large pileate tongue-shaped fruiting bodies on various substrates and are common in temperate regions. Despite their wide distribution, most have been described from North America and Southwest China (Zhuang 1998, Hustad et al. 2011, Wang et al. 2011).

Spathulariopsis (Cudoniaceae) is a monotypic genus. Also known as velvet-foot fairy fan, *S. velutipes* forms a yellowish to brownish yellow laterally compressed fan- or spatula-like head on a narrow brownish stem, needle-shaped hyaline occasional septate ascospores, and 8-spored asci.

Trichoglossum (Geoglossaceae) contains 19 species (Kirk et al. 2008), commonly known as black earth-tongues. *Trichoglossum* taxa are usually characterized by club-shaped brownish black fruiting bodies, smooth or velvety stipes, 7–15-septate smooth dark ascospores, large 4–8-spored asci, and a positive reaction of the apical ascus pore in Meltzer's reagent (Arora 1986, Hansen & Knudsen 2000).

According to the literature on Turkish macrofungi (Solak et al. 2007; Sesli & Denchev 2008; Akata et al. 2011, 2012; Allı et al. 2011), only two earth-tongues,

Leotia lubrica (Scop.) Pers. and *Spathularia flavida* Pers., have thus far been registered from Turkey.

Here we report the first Turkish collections of two additional earth-tongue genera, *Spathulariopsis* and *Trichoglossum*.

Materials & methods

Specimens were collected from Uzungöl Nature Park in 2011. The samples were photographed in the field and relevant morphological and ecological characteristics of the samples were recorded. Macroscopic and microscopic data were obtained using standard mycological techniques, and the specimens were identified by consulting Arora (1986), Hansen & Knudsen (2000), Breitenbach & Kränzlin (1984), and Jordan (2004). The specimens are kept in the herbarium of Ankara University, Ankara, Turkey (ANK).

Taxonomy

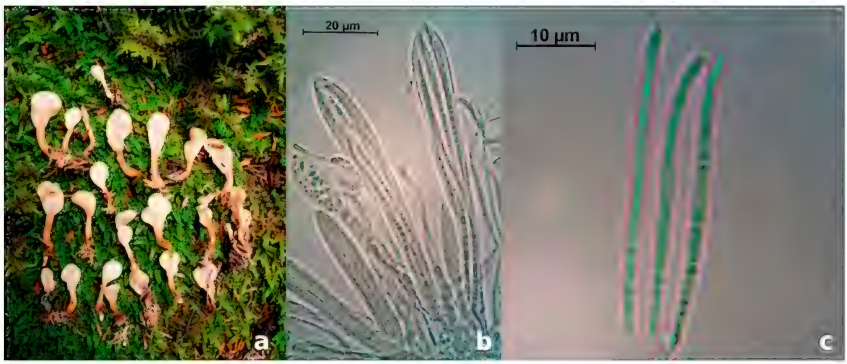

FIGURE 1. *Spathulariopsis velutipes* (Akata 4189): a. ascocarps; b. asci; c. ascospores.

Spathulariopsis velutipes (Cooke & Farl. ex Cooke) Maas Geest., Proc. K. Ned. Akad. Wet., Ser. C, 75: 254. 1972 FIG. 1

ASCOCARP 20–60 mm tall, spathulate, with a distinct head and stipe; HEAD flattened or laterally compressed, pale yellow to brownish yellow or cream colored and usually wrinkled or fairly smooth; FLESH whitish and insubstantial; STIPE 10–30 × 5–8 mm, compressed and flattened at apex, cylindrical below, ochre to reddish-brown and minutely velvety, with a typical orangish basal mycelium.

ASCI 70–90 × 8–12 µm, clavate, multiseriate eight spored, inamyloid; PARAPHYSES up to 2 µm broad, slender, tips spirally curved; ASCOSPORES 35–40 × 2–2.5 µm, needle-shaped, hyaline, smooth, sometimes septate, usually with several droplets.

SPECIMEN EXAMINED — **TURKEY. TRABZON**, Çaykara, Uzungöl Nature Park, in spruce (*Picea orientalis* (L.) Pererm.) forest, 40°37′N 40°18′E, 1420 m, 23.10.2011, Akata 4189 (ANK).

Comments — *Spathulariopsis velutipes* could be confused with *Spathularia flavida* due to its similar morphology. *Spathularia flavida* has a paler (whitish) smooth stipe, while *S. velutipes* has a minutely fuzzy, brownish stipe, orange basal mycelium, and shorter spores (Phillips 2005; Kuo 2005).

Trichoglossum hirsutum (Pers.) Boud., Hist. Classific. Discomyc. Europe: 86. 1907,
var. *hirsutum* Fig. 2

Ascocarp 40–70 mm tall, clavate, fertile head 10–15 × 5–8 mm, spathulate to clavate, tapering into the slender compressed and grooved stipe; stipe 2–3 mm thick, cylindrical. Surface dry, black to brownish black with velvety hairs; Flesh black and brittle.

Asci 170–210 × 20–25 µm, cylindrical to clavate, eight spored, spores parallel in ascus, amyloid; paraphyses filiform, curved, slightly swollen at the tips. ascospores 120–140 × 6–7 µm, bacilliform, smooth, brown, with 15 septa. setae up to 170 µm, thick walled, dark brown to black.

Specimen examined — **TURKEY. Trabzon**, Çaykara, Uzungöl Nature Park, on soil, near mixed spruce (*Picea orientalis*) and beech (*Fagus orientalis* Lipsky) forest, 40°36′N 40°16′E, 1470 m, 27.08.2011, Akata 4077 (ANK).

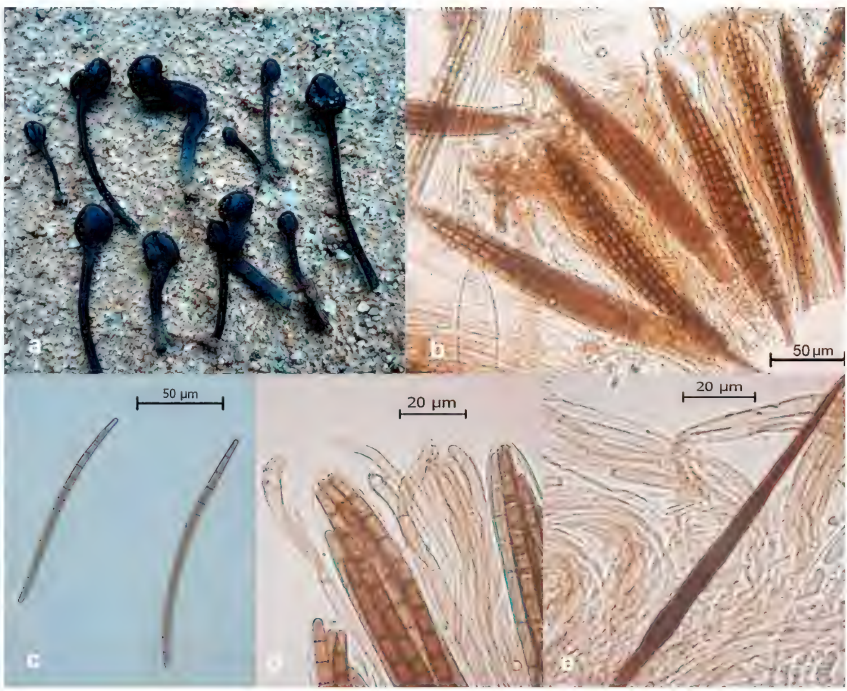

Figure 2. *Trichoglossum hirsutum* (Akata 4077):
a. ascocarps; b. asci; c. ascospores; d. paraphyses; e. seta.

Comments —*Trichoglossum* representatives differ from other black earth-tongues primarily by the velvety hair covering their ascocarps. Morphologically it is difficult to distinguish *Trichoglossum* taxa from each other. *Trichoglossum hirsutum* and *T. tetrasporum* Sinden & Fitzp. can be distinguished from other *Trichoglossum* species only at maturity by their 15-septate bacilliform spores. Asci of *T. hirsutum* contain eight spores while those of *T. tetrasporum* contain four (Hansen & Knudsen 2000, Breitenbach & Kränzlin 1984).

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Two new species of *Septobasidium* (*Septobasidiaceae*) from Yunnan Province in China

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Abstract —Two new species, *Septobasidium fissuratum* on *Castanea* sp. associated with *Pseudaulacaspis* sp. and *Septobasidium heliciae* on *Helicia nilagirica* associated with *Lepidosaphes* sp., are described.

Key words —*Pucciniomycetes*, *Septobasidiales*, taxonomy

Two new species of *Septobasidium* were recently discovered from Yunnan Province. These specimens, collected by Dr. Shuanghui He, are described below.

Septobasidium fissuratum Wei Li bis & L. Guo, **sp. nov.** Figs 1–7

Fungal Name FN 570061

Differs from *Septobasidium meridionale* by its larger basidia.

Type: China, Yunnan Province, Longchuan, Longba, alt. 1000 m, on *Castanea* sp. (*Fagaceae*), associated with *Pseudaulacaspis* sp. (*Diaspididae*), 31.X.2012, S.H. He YN08 (HMAS 244419, **holotype**).

Etymology: The epithet refers to the fissures of basidiomata.

Basidiomata on branches, 10–14 cm long, 2.5–6 cm wide, pale cinnamon-brown or cinnamon-brown; margin determinate, surface smooth, with many fissures at maturity. In section 600–1100 μm thick, composed of 3–4 layers. Subiculum brown, 30–50 μm thick. Pillars brown, 100–180 μm high, 30–100 μm wide, branched outwards to form a hyphal layer 350–700 μm high, then forming 20–40 μm thick horizontal layer, from which the fungal hyphae successively producing hyphal layers at maturity. Hymenium hyaline or brownish, 60–100

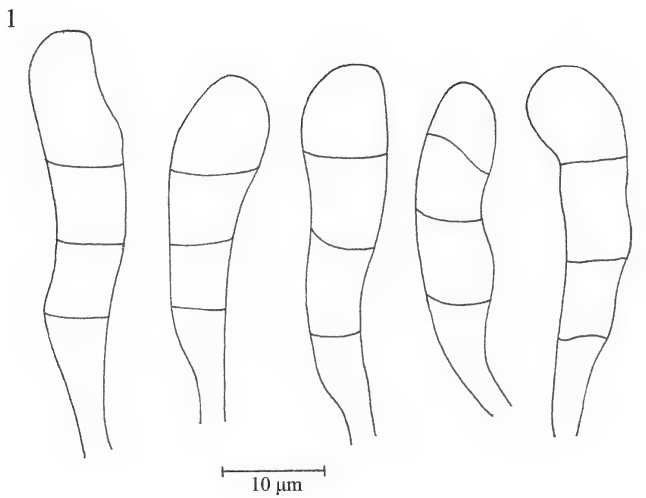


Fig. 1. *Septobasidium fissuratum* (HMAS 244419, holotype). Basidia.

μm thick. Basidia arising directly from the hyphae without a probasidial cell, cylindrical, straight or slightly curved, 4-celled, 32–45 × 6–9 μm, hyaline or brownish. Basidiospores not seen. Haustoria consisting of irregularly coiled hyphae.

Comments: *Septobasidium fissuratum* is similar to *S. meridionale* C.X. Lu & L. Guo, but the latter has fewer fissures on the basidiome surface, an inconspicuous horizontal layer in the section, and smaller basidia, 27–36 × 7–9.5 μm (Lu & Guo 2010b).

Septobasidium heliciae Wei Li bis & L. Guo, **sp. nov.** Figs 8–14

Fungal Name FN 570062

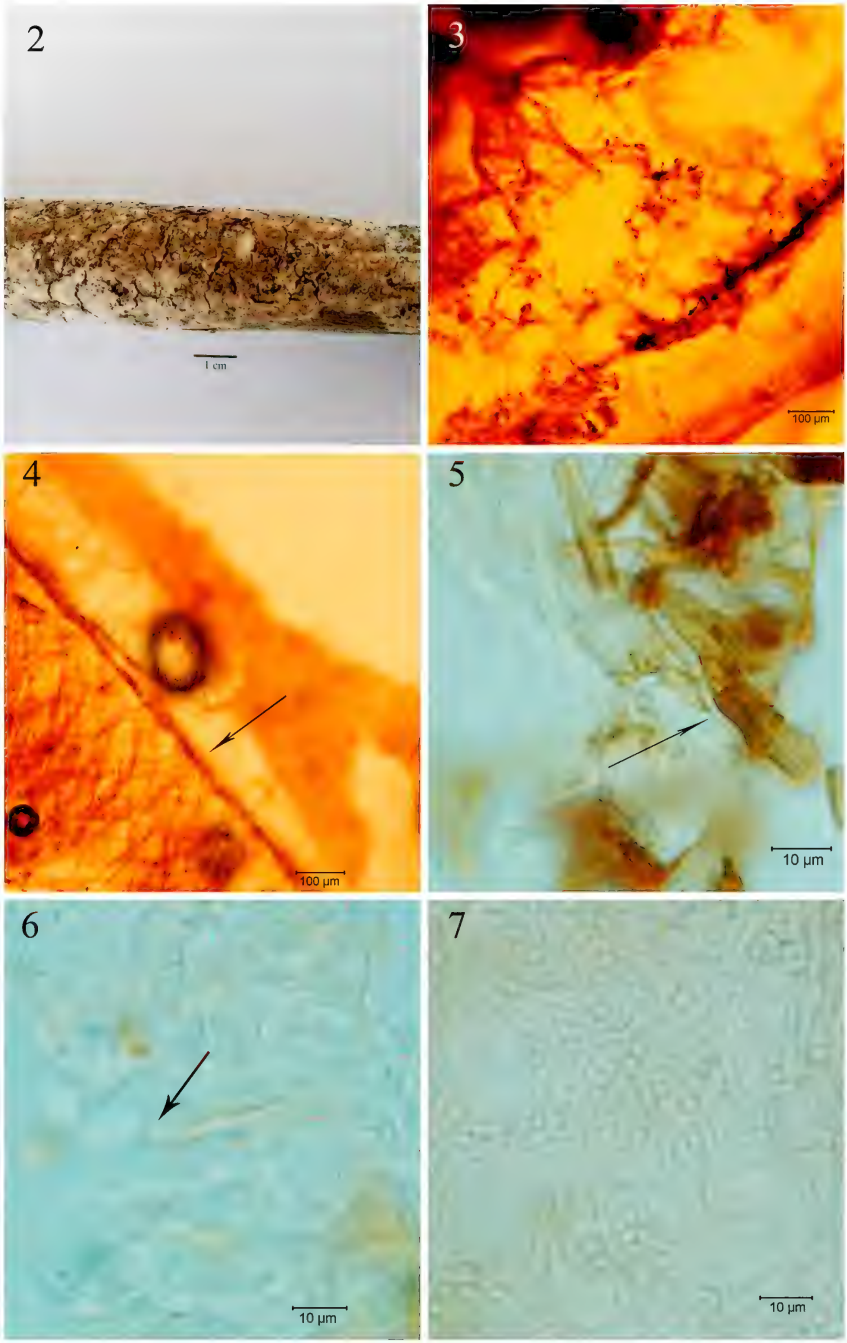
Differs from *Septobasidium septobasidioides* by its wider pillars and smaller basidia.

Type: China, Yunnan Province, Yingjiang, Tongbiguan Nat. Res., alt. 1250 m, on *Helicia nilagirica* (*Proteaceae*), associated with *Lepidosaphes* sp. (*Diaspididae*), 29.X.2012, S.H. He YN07 (HMAS 244418, **holotype**).

ETYMOLOGY: The epithet refers to the substrate plant genus.

Basidiomata on branches, 4–13 cm long, 4–5 cm wide, white or pale cinnamon-brown; margin determinate, surface smooth. In section 750–1100 μm thick.

Figs. 2–7 *Septobasidium fissuratum* (HMAS 244419, holotype). 2. Basidiomata on branch. 3–4. Sections of basidiomata (arrow shows horizontal layer). 5–6. Basidia (arrows). 7. Haustoria.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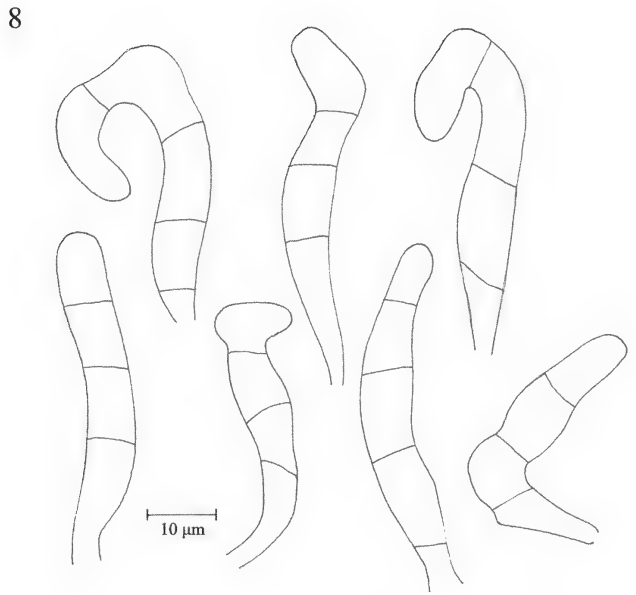


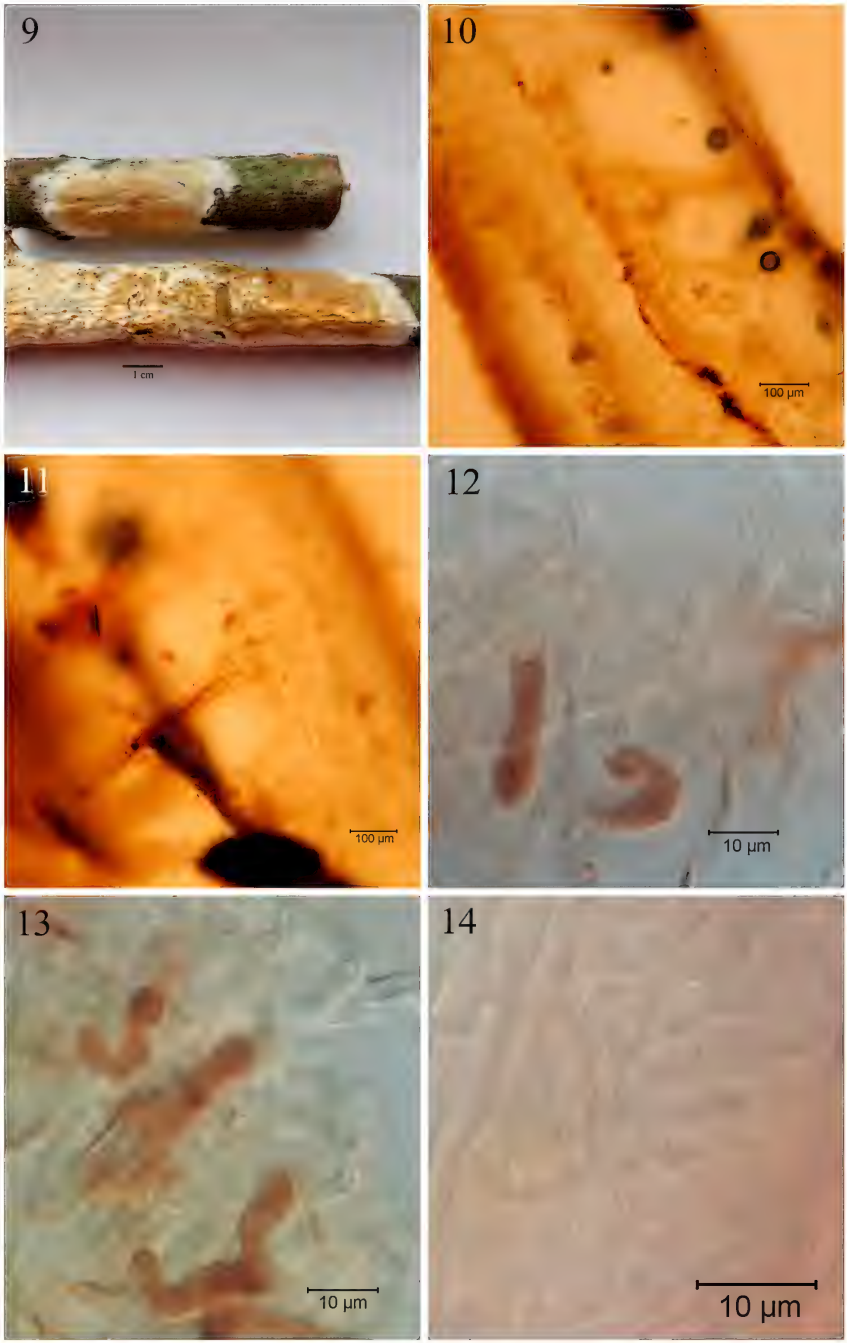
Fig. 8. *Septobasidium heliciae* (HMAS 244418, holotype). Basidia.

Subiculum brown, 10–20 μm thick. Pillars brown, 120–600 μm high, 30–120 μm wide, branched outwards to form a hymenial layer. From the old hymenium successively forming new hyphal layer or pillars and hymenium. Hymenium hyaline or brownish, 80–110 μm thick, with irregularly arranged hyphae. Basidia arising directly from the hyphae without a probasidial cell, cylindrical, straight or curved, 4-celled, 30–45 × 5–6.5 μm, hyaline or brown. Basidiospores not seen. Haustoria consisting of coiled hyphae.

Comments: *Septobasidium heliciae* is similar to *S. septobasidioides* (Henn.) Höhn. & Litsch., but the latter has basidiomata with lobed wings, slender pillars (10–25 μm wide), and larger basidia (40–55 × 8.4–10 μm; Couch 1938).

Including the two new species reported in this paper, 51 *Septobasidium* species have now been reported in China (Sawada 1933, Couch 1938, Teng 1963, Tai 1979, Kirschner & Chen 2007, Lu & Guo 2009a,b,c, 2010a,b,c, 2011, Lu et al. 2010, Chen & Guo 2011a,b,c,d, 2012a,b,c).

Figs. 9–14. *Septobasidium heliciae* (HMAS 244418, holotype). 9. Basidiomata on branches. 10–11. Sections of basidiomata. 12–13. Basidia. 14. Haustoria.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Septobasidium hoveniae sp. nov. and *S. rhabarbarinum* new to China

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Abstract —A new species, *Septobasidium hoveniae* on *Hovenia acerba* associated with *Pseudaulacaspis* sp., is described. *Septobasidium rhabarbarinum* on *Quercus pannosa* is new to China.

Key words —*Pucciniomycetes*, *Septobasidiales*, *Septobasidiaceae*, taxonomy

A new species of *Septobasidium* was discovered in the famous Huangshan Scenic Area. Owing to the mild and humid climate and plentiful rainfall, Huangshan Mountain is provided with a great biodiversity. Among *Septobasidium* specimens collected by the fourth author a new species was discovered and is described below:

Septobasidium hoveniae Wei Li bis, S.Z. Chen, L. Guo &Yao Q. Ye, **sp. nov.** Figs 1–4

Fungal Name FN570057

Differs from *Septobasidium henningsii* by its thinner hymenium.

Type: China, Anhui Province, Huangshan Scenic Area, Wenquan, alt. 640 m, on *Hovenia acerba* Lindl. (*Rhamnaceae*), associated with *Pseudaulacaspis* sp. (*Diaspididae*), 31.V.2012, Y.Q. Ye 5 (HMAS 252321, **holotype**).

ETYMOLOGY: The epithet refers to the substrate plant genus.

Basidiomata on branches, 10 cm long, 5 cm wide, cinnamon-brown; margin determinate, surface smooth, cracked at maturity, partly peeling off, emerging brown pillars. In section 600–1800 µm thick. Subiculum brown, 50–70 µm thick. Pillars brown, at first straight, later curved and confluent, and then

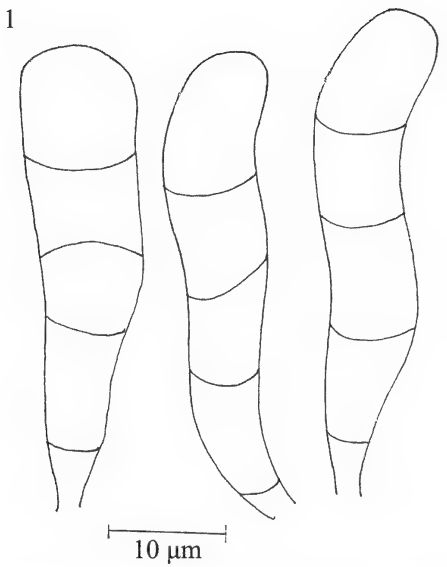

Fig 1. *Septobasidium hoveniae* (HMAS 252321, holotype). Basidia.

separable, 200–1500 μm high, 20–120 μm wide. Hyphal layer brown, 80–150 μm high. Hymenium hyaline or brownish, 100–110 μm thick. Basidia arising directly from the hyphae without a probasidial cell, cylindrical, straight or slightly curved, 4-celled, 35–45 × 7.5–9 μm, hyaline. Basidiospores not seen. Haustoria consisting of hyphae.

Comments: *Septobasidium hoveniae* is similar to *S. henningsii* Pat., which differs by having numerous fissures on the basidiome surface and a thicker and stratified hymenium, 60–400 μm high (Couch 1938).

Septobasidium rhabarbarinum (Mont.) Bres., Annls. Mycol. 14(3/4): 240, 1916.
Figs 5–8

Basidiomata on branches, 2–8 cm long, 1–3 cm wide, cinnamon-brown or old golden; margin determinate, surface smooth, with numerous cracks at maturity. In section 320–700 μm thick. Subiculum brown, 20–30 μm thick. From subiculum forming brown hyphal layer, broken by channels at base. Hymenium stratose, brown or brownish, 140–150 μm thick. Basidia arising

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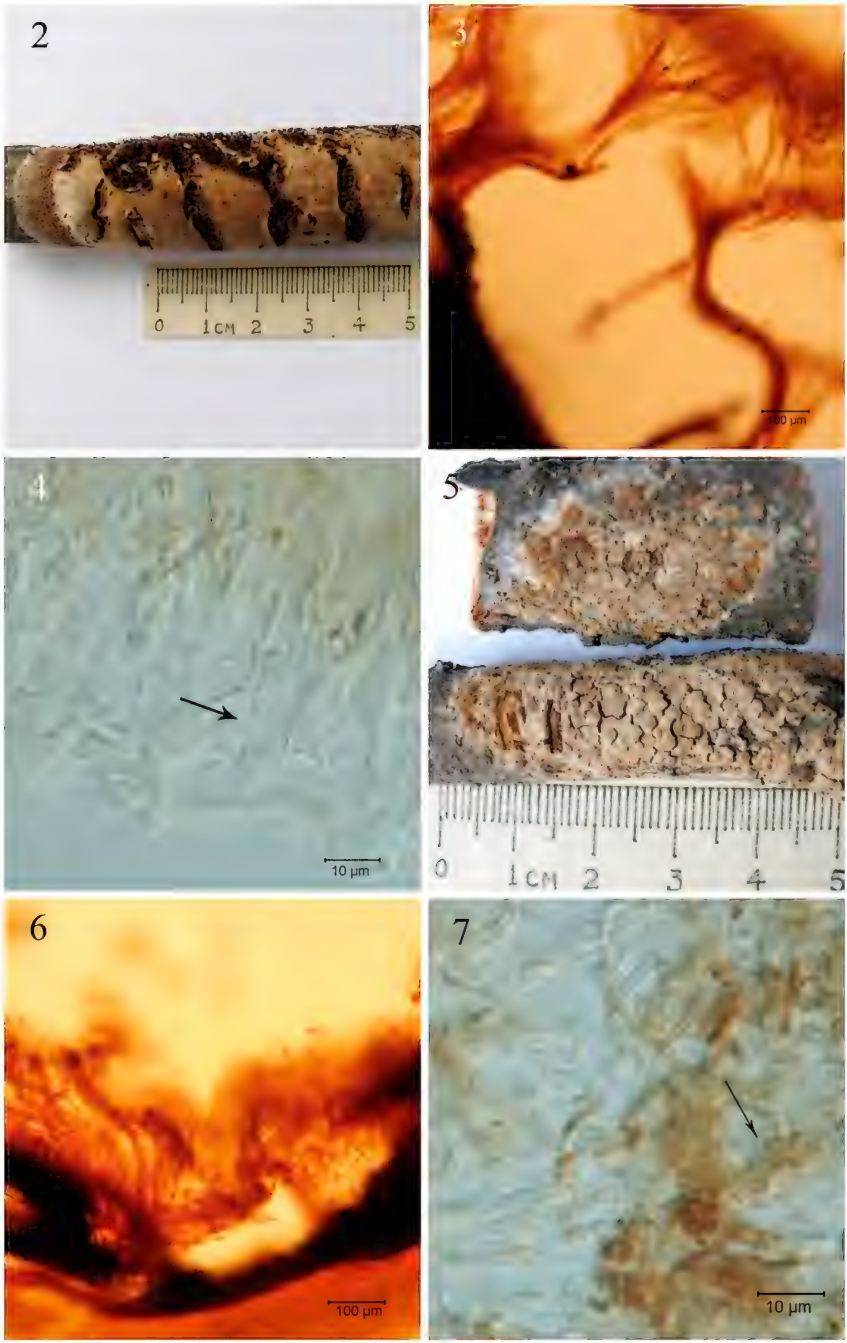
Figs 2–4. ***Septobasidium hoveniae*** (HMAS 252321, holotype). 2. Basidioma on branch; 3. Section of basidioma; 4. Basidium (arrow). Figs 5–7. ***Septobasidium rhabarbarinum*** (HMAS 251990). 5. Basidiomata on branches; 6. Section of basidioma; 7. Basidium (arrow).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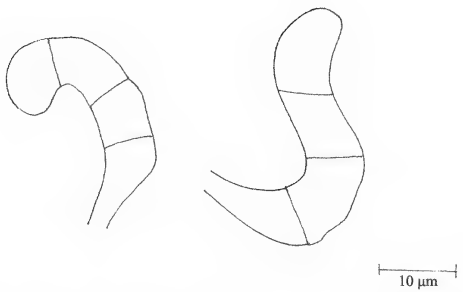

Fig 8. *Septobasidium rhabarbarinum* (HMAS 251990). Basidia.

directly from the hyphae without a probasidial cell, cylindrical, curved, 4-celled, 33–38 × 6–7 µm, brown. Basidiospores not seen. Haustoria consisting of irregularly coiled hyphae.

specimens examined: **CHINA, Sichuan:** Yanyuan, Luguhu, alt. 2700 m, on *Quercus pannosa* Hand.-Mazz. (*Fagaceae*), 14. IX. 2010, Y.F. Zhu & L. Guo 342 (HMAS 251990), Y.F. Zhu & L. Guo 340 (HMAS 263425).

Comments: Our materials are much like *S. rhabarbarinum* described by Couch (1938), except that the basidia are shorter (33–38 × 6–7 µm vs. 50–68 × 6.3–7.1 µm).

Including the two species reported in this paper, 53 *Septobasidium* species have now been reported in China (Sawada 1933, Couch 1938, Teng 1963, Tai 1979, Kirschner & Chen 2007, Lu & Guo 2009a,b,c, 2010a,b,c, 2011, Lu et al. 2010, Chen & Guo 2011a,b,c,d, 2012a,b,c, Li & Guo 2013).

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Validation and typification of *Gyroporus purpurinus*

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Abstract — *Gyroporus purpurinus* is a fairly well known eastern North American bolete that has never been validly published. It is validated and typified here.

Key words — *Boletales*, nomenclature

Introduction

Gyroporus purpurinus is a small bolete in the *Sclerodermatineae* distributed throughout the eastern United States and adjacent Mexico and Canada. Though well depicted on the Internet (Kuo 2003) and in popular field guides (Lincoff 1981, Phillips 1991, Bessette et al. 2000), the taxon has never been validly published.

As noted by Both (1993), Snell (1936), who first documented the fungus as "*Boletus castaneus* f. *purpurinus*," did not provide a Latin description or designate a type. This did not constitute valid publication, since a Latin description was required from 1935 to 2011 (McNeill et al. 2012: Art. 39.1). Singer (1945), who elevated the form to species level, combined it into *Gyroporus*, assuming Snell's "f. nov." to be valid. Thus, Singer's combination was also invalid. Smith & Thiers (1971) incorrectly implied that a 1915 collection by Kauffman that was sent to Snell (almost certainly BPI 782366) was the holotype, assuming a collection in their possession was an isotype (presumably MICH 4998). The University of Michigan Fungus Collection Database indicates that MICH 4998 is a syntype (quod.lib.umich.edu/f/fung1ic/ – accessed April 17th, 2013), but this is not possible because Snell (1936) did not reference any collections. Confusingly, the box of BPI 782371 is marked with a printed label that reads "type" (nt.ars-grin.gov/fungaldatabases/ – accessed April 17th, 2013). It is unclear whether this was marked by Snell or someone else.

Nevertheless, the taxon constitutes a discrete species. *Gyroporus purpurinus* is validated and typified here.

Materials & methods

Dried specimens were examined macroscopically with an AO Spencer stereomicroscope and microscopically with an Olympus BH-2 microscope. Dissections for microscopic study were prepared on slides with either water or 3% potassium hydroxide. In the description of spores, Q is the mean length/width quotient. The description of the taxon was drawn from previous descriptions in relevant literature (Snell 1936, Singer 1945, Bessette et al. 2000), aspects of which were modified based on study of specimens cited here. The color terms "reddish-purplish" and "vinaceous red to burgundy" are taken from Snell (1936) and Bessette et al. (2000), respectively.

Taxonomy

Gyroporus purpurinus Singer ex Davoodian & Halling, **sp. nov.**

MycoBank 804040

"*Boletus castaneus* f. *purpurinus*" Snell, Mycologia 28: 465 (1936), nom. inval. (no Latin description).

"*Gyroporus purpurinus*" Singer, Farlowia 2: 236 (1945), nom. inval. (no Latin description).

Distinguished from other members of *Gyroporus* by its distinct reddish-purplish pileus and stipe surfaces and the relative rarity of clamp connections.

Type: United States, New York, Glens Falls Watershed, near Lake George, 24 Aug 1932, col. W. H. Snell (**holotype**, BPI 782371).

Pileus 1–9 cm, convex to plane with even margin, somewhat velutinate to subtomentose, sometimes slightly rimose to finely areolate in age, "reddish-purplish"/"vinaceous red to burgundy"/various intergrading shades of purple and red. Hymenophore adnexed to free, white then becoming yellow with maturity, unchanging on injury or exposure; pores 1–4 per mm, tube depth 5–8 mm. Stipe 3–6 cm long, 2.5–10 mm thick, equal or tapering upward, often hollowing in age, somewhat velutinate, exhibiting colors within the same range of the pileus, lacking an annular zone. Context white, unchanging upon exposure or injury. Spore print bright yellow.

Spores 8–11(–12) × (4–)4.8–7 µm, Q = 1.8, nearly ovoid to ellipsoid, sometimes appearing subreniform, appearing smooth, hyaline to yellow, sometimes appearing with a slightly thickened wall, sometimes appearing with hilar appendage. Basidia 21–30 × 8–12.2 µm, 4-spored. Hymenophoral cystidia versiform, 23–50 × 3.3–8 µm. Tube trama divergent. Pileipellis a collapsing trichodermium; terminal cells fusoid-ventricose to ampullaceous, 40–120 × 5–20 µm; pigmentation at times apparent. Stipitipellis hyphae oriented in various directions; pigmentation at times apparent. Clamp connections infrequent to rare.

Distribution: Florida to New England, west to Minnesota and northeast Mexico. Reported from southeast Canada (mycoportal.org/portal/imagelib/ – accessed April 17th, 2013). Phenology: July–October. Presumed mycorrhizal symbionts: *Quercus*, *Pinus*.

Other specimens examined – **UNITED STATES**, **Michigan**, Ann Arbor, 15 Aug 1915, col. C. H. Kauffman (BPI 782366). **Massachusetts**, Conway, Pine Hill Road, 30 Aug 1960, col. H. E. Bigelow with M. E. Bigelow (NY 48358). **Georgia**, Athens, University of Georgia Botanical Garden, 25 Aug 1978, col. H. E. Bigelow given to R. E. Halling (NY 48357). **North Carolina**, Swain Co., Great Smokey Mountains National Park, Indian Creek, 1 Aug 1992, col. R. E. Halling (NY 48356).

Comments – For illustrations of microscopic features see Singer (1945) and Smith & Thiers (1971).

Acknowledgments

We appreciate the insight of Dr. Andrew Minnis regarding nomenclatural points in this paper. Dr. Timothy Baroni and Dr. Brandon Matheny are thanked for their helpful reviews of this manuscript. Dr. Shaun Pennycook is thanked for his essential nomenclatural review of this paper.

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Golovinomyces clematidis sp. nov. from China

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Abstract — *Golovinomyces clematidis* (*Erysiphaceae*), a new species found on *Clematis aethusifolia* (*Ranunculaceae*) in Inner Mongolia, China, is described, illustrated, and compared with morphologically similar species of the *G. cichoracearum* s. lat. complex.

Key words — *Erysiphales*, powdery mildews, taxonomy

Introduction

Some interesting specimens of powdery mildew on *Clematis aethusifolia* (*Ranunculaceae*) were collected in Ulanqab City, Inner Mongolia Autonomous Region, in northern China in 2005 and 2012. This was unusual as the first ranunculaceous host plant reported to be infected by a species of *Golovinomyces* (U. Braun) Heluta (Braun 1987; Chen et al. 1987; Nomura 1997; Shin 2000; Liu 2010; Braun & Cook 2012). Based on its ellipsoid-ovoid, doliiform to subcylindrical, non-limoniform conidia as well as relatively short and cylindrical conidiophores, this fungus should be treated as a new species of *Golovinomyces* sect. *Golovinomyces* (Braun & Cook 2012). It is morphologically close to the *G. cichoracearum* (DC.) Heluta s. lat. complex, a widespread powdery mildew fungus on hosts of *Asteraceae* (Braun & Cook 2012).

Materials & methods

The fungal materials were mounted in distilled water and examined using standard light microscopy without staining. For each collection, 100 conidia and related structures were measured in water. The specimens were deposited in the Mycological Herbarium of Chifeng University, Inner Mongolia, China (CFSZ), the Mycological Herbarium of the Institute of Microbiology, Academia Sinica, Beijing, China (HMAS), and the Herbarium of Martin-Luther-University, Halle (Saale), Germany (HAL).

Taxonomy

Golovinomyces clematidis T.Z. Liu & Jing Wen, **sp. nov.** Fig. 1

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Differs from *Golovinomyces cichoracearum* s. str. and *G. orontii* by its longer conidiophores, which are consistently straight at the base of foot-cells, and its smaller conidia.

Type: On living leaves of *Clematis aethusifolia* Turcz. (*Ranunculaceae*): China, Inner Mongolia, Ulanqab City, Xinghe County, Datongyao, 24 Aug. 2012, T.Z. Liu, Z.H. Jia & D.N. Su (**Holotype**, CFSZ 5940; **isotypes**, HMAS 244659, HAL 2591 F).

Etymology: derived from the generic name of the host plant.

Mycelia on stems and leaves amphigenous, effuse or forming irregular white patches, often occupying the whole leaf surface, persistent or subevanescent. Hyphae 4.5–7.5 µm wide, hyaline to yellowish, thin-walled, smooth or rarely verruculose. Appressoria distinctly nipple-shaped. Conidiophores erect, 100–200 µm long, foot-cells cylindrical, straight or occasionally somewhat flexuous, 55–100 × 8–12.5 µm, followed by 2–3 shorter cells. Conidia in chains (catenescent), ellipsoid-ovoid, doliiform to subcylindrical, relatively small, 20–34 × 10–15 (average 25 × 13) µm. Chasmothecia scattered to gregarious, dark brown, subglobose, 80–130(–140) (average 105) µm diam. Peridium cells irregularly polygonal to daedaleoid, 7.5–25 µm diam. Appendages numerous, about 15–70 or more, usually equatorial and in the lower half of the chasmothecium, sometimes reaching the upper half, mycelium-like, often interlaced with mycelia and with each other, mostly unbranched or rarely irregularly branched one time, flexuous, sometimes tortuous to geniculate, (0.5–)1–5 times as long as the chasmothecial diam., (50–)100–550 µm long, (2.5–)4–10 µm wide, thin-walled, smooth, somewhat rough near the base, (0–)1–9-septate, brown throughout or in the basal half, paler towards the apex, hyaline in the upper portion. Asci 8–22, oval, oblong-oval or irregularly shaped, mostly stalked, sometimes sessile, 40–75 × 20–40 (average 59 × 29.5) µm. Ascospores 2(–3) per ascus, ellipsoid or ovoid, yellowish, (15–)20–25 (–30) × (10–)12.5–15(–17.5) (average 23 × 14) µm.

Additional specimen examined: On living leaves of *Clematis aethusifolia*: **China, Inner Mongolia**, Ulanqab City, Jining District, Laohushan, 16 Oct. 2005, T.Z. Liu (CFSZ 05392).

Comments: *Golovinomyces clematidis* is the first *Golovinomyces* species to be collected on a host belonging to the *Ranunculaceae*. Hence, there are no comparable species on allied hosts. *Golovinomyces clematidis* is morphologically similar to species of the *G. cichoracearum* s. lat. complex including their chasmothecium size, appendage number and characteristics, and number and size of asci and ascospores (Braun & Cook 2012). However, species of this complex are specialized and confined to certain hosts belonging to particular tribes of the *Asteraceae*, i.e., they are characterized by a close co-evolution with their hosts (Matsuda & Takamatsu 2003, Braun & Cook 2012). The plurivorous *G. orontii* (Castagne) Heluta is one exception that might have the potential to cause powdery mildew on *Clematis*, but it is easily distinguished by the

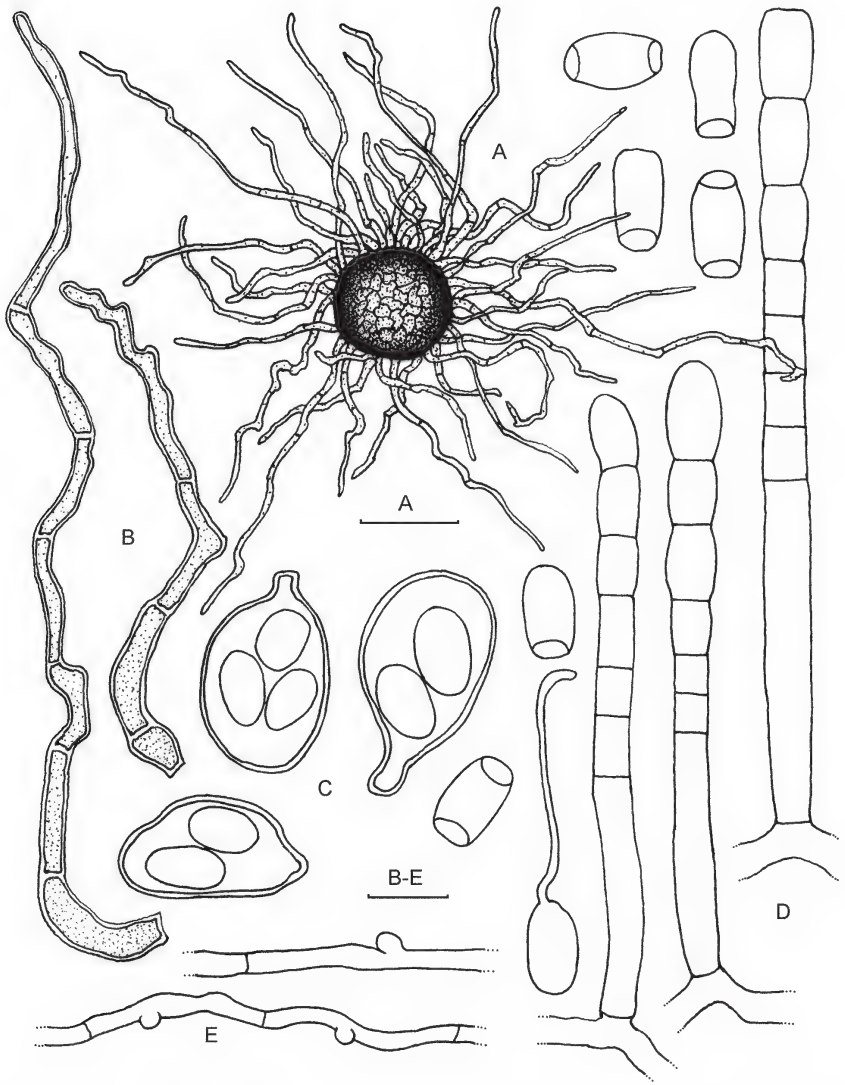

FIGURE 1. *Golovinomyces clematidis* (holotype). A. Chasmothecia; B. Appendages; C. Asci and ascospores; D. Conidiophores and conidia; E. Hyphae and appressoria. Scale bars: A = 100 μm; B–E = 25 μm. T.Z. Liu del.

frequently curved base of its conidiophore foot-cells (Liu 2010, Braun & Cook 2012). *Golovinomyces cichoracearum* s. str. also differs in its curved to sinuous conidiophore foot-cells that are both shorter and wider ((30–)40–80 × (9–)10–

15(–18) μm) as well as much larger conidia (25–42 × 14–23 μm). The conidia of *G. clematidis* differ significantly from all other species of the *G. cichoracearum* complex. One specimen of *G. clematidis* (CFSZ 05392) has previously been identified as and confused with *Erysiphe aquilegiae* var. *ranunculi* (Grev.) R.Y. Zheng & G.Q. Chen (Liu 2010).

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Four noteworthy hyphomycetes from indoor environments

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Abstract — Four interesting hyphomycetes (*Nalanthamala vermoesenii*, *Parascedosporium putredinis*, *Stachybotrys elegans*, *Triadelphia australiensis*) have been collected from indoor fungal investigations. Among these fungi, *Triadelphia australiensis* represents a new record for Canada and the USA. *Parascedosporium putredinis* and *Stachybotrys elegans* are reported for the first time from indoor environments.

Key words — anemophilous, microfungi, mold, residence

Introduction

Fungal diversity inside buildings is poorly understood. Although the list of fungi from indoor environments compiled from the databases at commercial laboratories is expanding to include new first records of fungi occurring indoors, no comprehensive list of indoor fungi exists. Recently, four noteworthy hyphomycete species found in indoor environments among samples collected from air and building materials during indoor mold investigations in the USA and Canada were submitted to our laboratories for analysis. We report on these indoor fungi here.

Materials & methods

Fungal isolates were grown on malt extract agar (MEA) at 25°C in the dark. *Parascedosporium putredinis* was also grown on corn meal agar (CMA), dichloran

glycerol agar (DG18), and potato dextrose agar (PDA). If culturing was not successful, tape lifts or a small scalpel were used to get fungal structures directly from the samples for microscopic observation. Conidiophores and conidia were mounted in 85% lactic acid. A staining agent, 0.1% lacto-fuchsin, was used to observe conidiogenous cells and septation of colorless conidia. All microscopic observations were made under Nomarski differential interference contrast optics. Photomicrographs were taken with an Olympus Microfire digital camera (Goleta, CA). Measurements of the fungal structures were statistically analyzed with Microsoft Office Excel 2010 with 95% confidence interval of means. The results were presented as ranges and mean ± standard deviation. Voucher specimens or cultures were deposited to China General Microbiological Culture Collection (CGMCC) and U.S. National Fungus Collections (BPI).

DNA extraction, sequencing, and sequence similarity analysis for *Nalanthamala vermoesenii* and *Parascedosporium putredinis* follow Li et al. (2008).

The partial DNA sequence transcribing the ribosomal spacer region (3' end of the 18S ssu), 5' end of the 28S ribosomal RNA units, and complete sequences for the 5.8S ribosomal RNA and internal transcribed spacers (ITS) 1 and 2) were submitted through the nucleotide MegaBLAST procedure (Zhang et al. 2000) via the NBCI web site (www.ncbi.nlm.nih.gov/blast/) using the non-human, non-mouse database. Sequence similarity searches and comparisons for *Nalanthamala vermoesenii* and *Parascedosporium putredinis* were conducted using MegaBLAST (NCBI).

The partial sequences transcribing the 18S and 28S ribosomal RNA units, and the complete sequences for the 5.8S ribosomal RNA and internal transcribed spacers (ITS) 1 and 2 have been placed in the GenBank database.

Taxonomy

Nalanthamala vermoesenii (Biourge) Schroers, Mycologia 97: 390 (2005) PLATE 1

≡ *Penicillium vermoesenii* Biourge, La Cellule 33: 230 (1923)

≡ *Gliocladium vermoesenii* (Biourge) Thom, The Penicillia: 502 (1930)

COLONIES 38–55 mm diam at 25°C after 5 d on MEA in the dark. Colony surface fine powdery to dusty, salmon to pink; aerial mycelium sparsely developed. Reverse flesh. CONIDIOPHORES dimorphic; penicillate and acremonium-like. Penicillate conidiophores up to 220 µm long, monoverticillate to quaterverticillate, 22–110 µm tall; metulae (6.5–)7.7–14(–19) × (2–)2.5–3.5 (–4) µm (n = 35); phialides cylindrical with a narrowed apex, (8–)10–16(–24) µm long, 1.5–3.5 µm wide at base and in the lower third, and 0.5–1.5 µm wide at the tip (n = 35). Acremonium-like conidiophores, unbranched or occasionally branched; phialides cylindrical or slightly narrowing toward the tip, 9–25(–40) × (1.5–)1.9–2.7(–3) µm, 1–2 µm wide at tip (n = 35). CONIDIA dimorphic: those on penicillate conidiophores 1-celled, ovoid or ellipsoidal, (3–)3.5–5.5(–7) × (2–)2.1–3.1(–4) µm (n = 30), in long, dry, persistent chains, in masses appearing salmon or pink in color; on acremonium-like conidiophores ellipsoidal or cylindrical (2–)4.8–10.5(–15) × (1.5–)1.7–2.8(–4) µm (n = 30), 1-celled, in liquid drops aggregated at the tip of phialides.

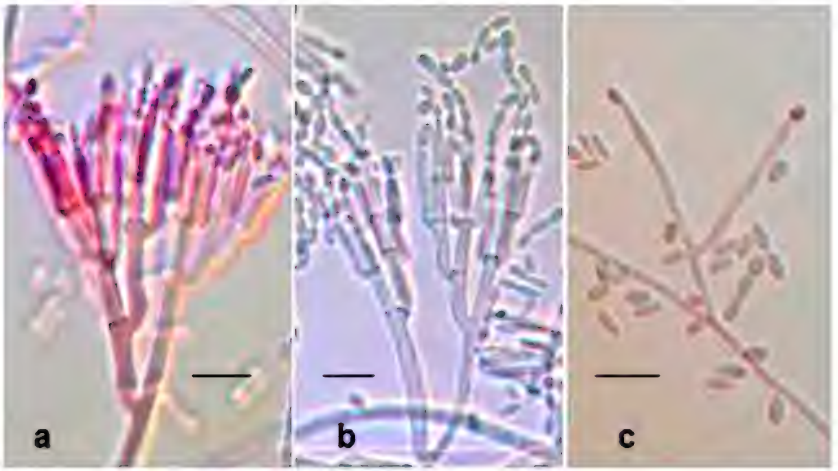


Plate 1. *Nalanthamala vermoesenii* (CGMCC3.15232). a–b. Penicillate conidiophores and conidia; c. *Acremonium*-like conidiophore and conidia. Scale bars = 10 µm.

Teleomorph. Unknown.

Material examined: **U.S.A. California**, Sacramento, in a residence, April 2005, anonymous (CGMCC3.15232; GenBank KC894849).

Distribution: Australia, Belgium, Congo, Czech Republic, Greece, India, Japan, New Zealand, Russia, Spain, South Africa, UK, USA, Uzbekistan. Mainly in warm temperate, Mediterranean, subtropical areas, also on hosts kept in greenhouses of other geographic areas (Subramanian 1956; Spaulding 1961; Carpenter et al. 1962; Holevas et al. 2000; Schroers et al. 2005).

Substrates/hosts: air, indoor environment, soil, on various *Arecaceae* (causing stem rot, pink rot, necrosis and blight), *Citrus medica*, *Psidium guajava* (Schroers et al. 2005).

Note: *Nalanthamala vermoesenii* has been found mainly in indoor environments on the west coast of North America, especially in California. It has also been reported indoors and outdoors from Florida and Hawaii (Raabe et al. 1981; Alfieri. et al. 1984; French 1989) but is not common in other areas of the U.S.A. It is a pathogen on palms, but it is occasionally found from indoor samples (Spaulding 1961). The species is often placed in *Gliocladium* or *Penicillium* due to sharing a similar morphology with those genera. Based on its phylogenetic relationships Schroers et al. (2005) transferred it to *Nalanthamala,* a genus erected by Subramanian (1956), typified by *N. madreeya* Subram., and currently comprising five species. *Nalanthamala vermoesenii* is morphologically similar to *N. psidii* (Sawada & Kuros.) Schroers & M.J. Wingf. on culture media. Its salmon to pink colonies on PDA and 1-celled conidia differentiate

N. vermoesenii from *N. psidii*, which has yellow colored colonies and 1–2 celled conidia (Schroers et al. 2005).

ITS sequence data of *N. vermoesenii* collected from indoor environments have a 100% match with the neotype isolate CBS 110893(= 5 MUCL 9504, Biourge 415, ex-type; GenBank AY554214) and a 95% match with the epitype isolate of *N. psidii* BPI 863661 (GenBank AY864836).

Parascedosporium putredinis (Corda) Lackner & de Hoog, IMA Fungus 2(1): 44 (2011) PLATE 2

≡ *Graphium putredinis* (Corda) S. Hughes, Can. J. Bot. 36: 770 (1958)

= *Parascedosporium tectonae* (C. Booth) Gilgado, Gené, Cano & Guarro, Int. J. Syst. Evol. Microbiol. 57: 2176 (2007)

COLONIES reaching 34 mm diam on MEA after 14 d at 25°C, velvety, dark gray and slightly raised at center, buff at edge, granulate due to presence of numerous synnemata, reverse pale gray at center; 49 mm diam on PDA, light gray, darker at center, granulate; reaching 53 mm, spreading out in a very thin layer of mycelium, almost transparent on CMA; no growth on DG18.

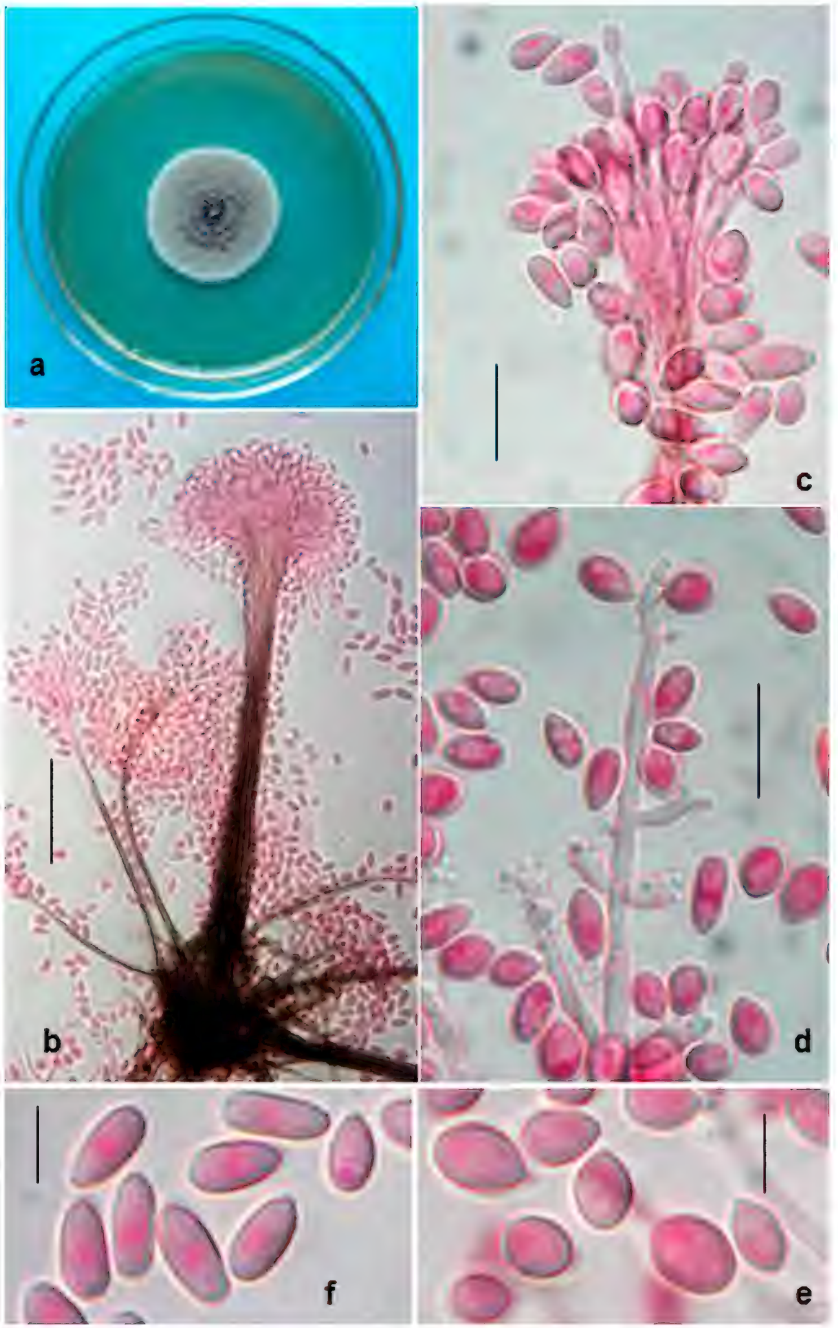
CONIDIOPHORES when solitary, simple, undifferentiated, with lateral conidiogenous cells, occasionally reduced to conidiogenous cells, or irregularly branched. CONIDIOGENOUS CELLS cylindrical or ampulliform, 10–25 × 2.0–2.5 µm, colorless, smooth, thin-walled, bearing 2–7 cylindrical denticles ≤1.2 µm long at apical area. CONIDIA obovoid or ellipsoidal, smooth, thick-walled, (4.3–)4.8–6.4 (–6.9) × (3.3–)3.6–4.4(–4.7) (mean = 5.6 ± 0.8 × 4.0 ± 0.4, n = 25) µm.

SYNANAMORPH: synnemata erect, up to 230 µm in height; stipe to 20 µm in width, dark grey, fanning out at apices and developing conidia in an opaque slimy mass. Conidiogenous cells percurrent, cylindrical, annellidic, lateral or terminal, colorless to subhyaline, smooth-walled, 10–22 × 2.0–2.5 µm. Conidia clavate or subcylindrical, smooth, (5.2–)5.9–7.3 (–7.9) × (2.6–)2.9–3.5(–3.7) (mean = 6.6 ± 0.7 × 3.2 ± 0.3, n = 25) µm. A small number of conidia developing directly from undifferentiated hyphae, lateral, subglobose to obovoid, brown, smooth and thick-walled, usually sessile, 6–7.5 × 5–6 µm.

MATERIAL EXAMINED: **USA**, **KENTUCKY**, Lexington, from particleboard under a kitchen sink in a residence, 27 June 2011, Chris Adkins (CGMCC3.15233; GenBank KC894850).

DISTRIBUTION: Australia, China, Cuba, Czech Republic, Italy, Jamaica, Japan, Malaysia, Madagascar, New Zealand, Nicaragua, Poland, The Netherlands, USA.

PLATE 2. *Parascedosporium putredinis* (CGMCC3.15233). a. Colony growing on MEA for 14 days at 25°C; b. Synnemata and conidia; c. Annellidic conidiogenous cells and conidia; d. Denticulate conidiogenous cells and conidia; e. Conidia developed from denticulate conidiogenous cells; f. Conidia developed from annellidic conidiogenous cells. Scale bars: b = 40 µm, c–d = 10 µm, e–f = 5 µm.


a
c
b
d
f
e

Substrates/hosts: *Actinidia deliciosa* (kiwifruit, leaf lesions), *Carex pseudocyperus(Mułenko, Majewski, Ruszkiewicz-Michalska 2008), Chrysalidocarpus lutescens, Echium* sp., *Elaeis* sp., *Iris pseudacorus, Pandanus boninensis, Quercus cerris* (living wood), *Schoenoplectus lacustris, Tectona grandis* (seed), *Theobroma cacao*, particle board, air, soil (Booth 1964; Liu 1977; Mercado Sierra et al. 1997; Kobayashi 2007; Mułenko et al. 2008; Zhang et al. 2008; Lackner & de Hoog 2011).

Note: Gilgado et al. (2007) erected *Parascedosporium* as a monotypic genus for *P. tectonae*. Lackner & de Hoog (2011) determined that *P. putredinis* was synonymous, so that the genus remains monotypic.

ITS sequence data of *P. tectonae* collected from indoors in Minnesota has 100% match with isolates from the ex-type of *P. tectonae* (GenBank AM749440), CBS 118694 (as *P. tectonae*; GenBank AM749735), and CMW352 (as *Graphium putredinis*; GenBank HQ335312.1) (Cruywagen et al. 2010). Sequence HQ335312.1 (from a C.T. Rogerson collection from a blackened agaric) is the only previous record of this fungus from the USA (Cruywagen et al. 2010). The 2011 Adkins collection is the first report of this fungus from an indoor environment.

Since this species is synanamorphic, developing both *Graphium* and *Scedosporium* anamorphs, care should be taken to avoid identifying this fungus as two different fungi when both anamorphs are present. This fungus is hydrophilic according to its growth on artificial media.

Stachybotrys elegans (Pidopl.) W. Gams, Compendium of Soil Fungi: 746 (1980)

Plate 3

= *Stachybotrys aurantia* G.L. Barron, Can. J. Bot. 40: 258 (1962)

Colonies on MEA reaching 30 mm diam at 21°C in 12 days; pink to salmon pink with light brown soluble pigment released into medium and conspicuously funiculose (downy) at center and velutinous at edge; radially sulcate on the edge; reverse dirty pink to pinkish brown. Conidiophores differentiated, single, determinate, erect, single, straight or flexuous, unbranched or alternately branched, colorless, smooth or minutely roughened, varying in length, (30–)42–64(–71) × (3.3–)3.5–4.1(–4.5) µm (n = 10), 0–5 septate for unbranched, up to 285 µm in length for alternately branched, tapering toward the apex, slightly enlarged at the apex and the base, terminally bearing a whorl of 2–7 phialides at the apex. Phialides determinate, discrete, subclavate, smooth, colorless, (6.7–)7–8.7(–10.9) × (3.8–)4–4.5(–4.9) µm (n = 20), with conspicuous collarettes. Conidia unicellular, acrogenous, colorless, smooth, ellipsoidal, (4.8–)6.6–8.2(–9.2) × (3.5–)3.9–4.7(–5.5) µm (n = 30), aggregated in slimy masses.

Material examined: **USA, New Jersey**, in a residence, 10 March 2008, anonymous (BPI 884211). Additional materials examined are listed in Li (2011).

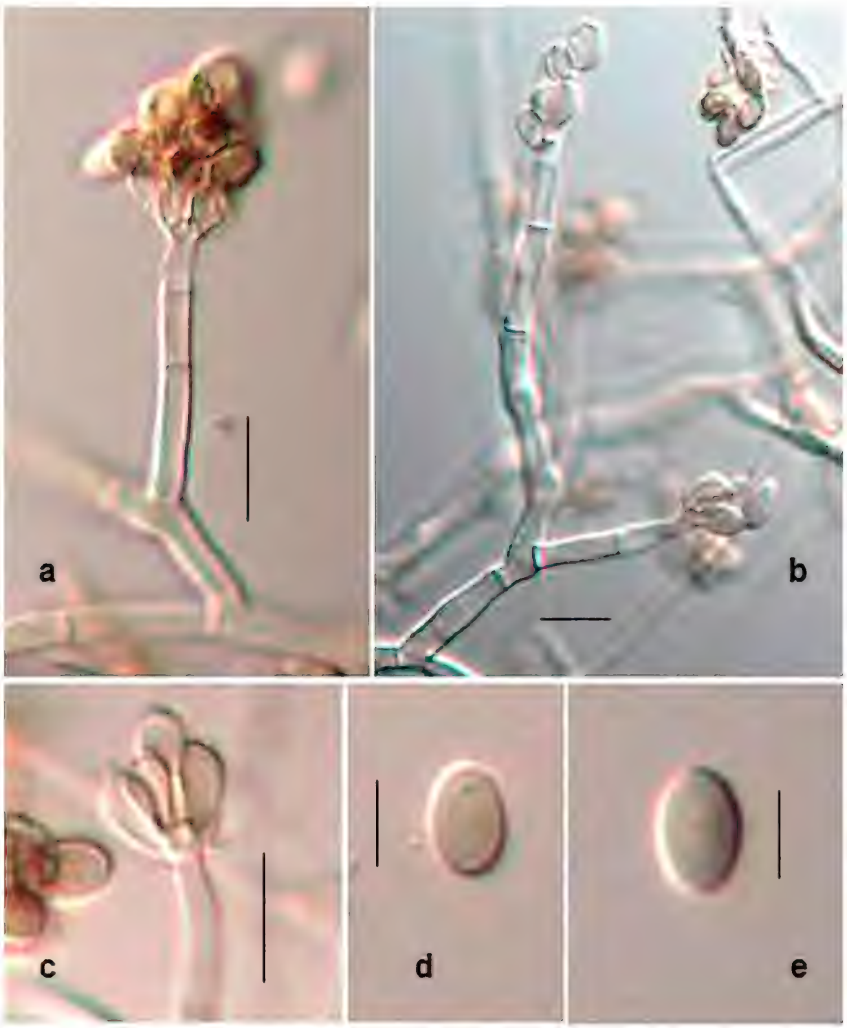


Plate 3. *Stachybotrys elegans* (BPI 884211). a. Conidiophore and conidia; b. Branched conidiophore and conidia; c. Phialides and conidia; d–e. conidium. Scale bars: a–c = 10 µm, d–e = 5 µm.

Distribution: Brazil, Cuba, Canada, China (as *Stachybotrys aurantia*, Wu et al. 2009), Egypt, Georgia, India, Kenya, Malawi, Malaysia, Poland, South Africa, USA, Vietnam.

Substrates/hosts: from soil, plants [*Agropyron repens* (rhizosphere), *Bambusa* sp. (on leaves.), *Betula alleghaniensis* (roots), *Dactylis glomerata*, *Linum usitatissimum*, *Panicum virgatum*, *Phaseolus vulgaris*, *Saccharum officinarum*,

Saccharum spontaneum, *Trifolium alexandrinum*, *Trifolium pratense*], and an isopteran insect.

Note: *Stachybotrys elegans* has been reported from soils and plant debris in North America (Orpurt 1954; Barron 1962; Morgan-Jones 1977; Ghimire et al. 2010). It is here reported for the first time from an indoor environment. Several *Stachybotrys* species (including *Memnoniella*) have previously been reported from indoor environments. Predominant indoor species are *S. chartarum* and *S. echinata* (≡ *M. echinata*), but *S. chlorohalonata*, *S. microspora*, and *S. yunnanensis* have also been reported.

Triadelphia australiensis B. Sutton, Sydowia 41: 339 (1989) Plate 4

[non *Triadelphia australiensis* Joanne E. Taylor et al., nom. illegit.]

Colonies on natural substrate thinly diffuse, sparse, dark brown. Mycelium initially immersed, finally superficial, sparse, of colorless to pale brown, irregularly branched, smooth, septate hyphae 2-3.5 µm wide. Conidiogenous cells integrated, terminal or lateral, holoblastic, monoblastic, colorless to pale brown, thin-walled, smooth, globose to ampulliform, more rarely lageniform, 3–5.5 × 3–4 µm, each with a single, short, unthickened denticle upon which a conidium is formed. Conidia aerogenous, solitary, dry, ellipsoidal, obovoid to broadly obovoid, smooth, thick-walled, 1-septate near the base, thickened, sometimes constricted at the septum, (8.2–)9.1–10.9 (–11.7) × (4.9–)5.6–6.8 (–7.3) (mean = 10 ± 0.9 × 6.2 ± 0.6, n = 30) µm, Q = 1.4–1.9 (mean = 1.6); two cells unequal in size and shape, the apical cell dark brown, thick-walled and the basal cell pale brown, with a small unthickened scar at the base.

Material examined, **CANADA, British Columbia**, Nanaimo, from plywood in an ice rink, 2 November 2011, Gordon Wedman (BPI 884210).

Distribution: Australia, Canada, USA.

Substrates/hosts: bark of unknown plant, unknown substrate in a residence, and plywood in an ice rink.

Note: *Triadelphia australiensis* represents a new record for Canada and the USA. It is morphologically very similar to *T. uniseptata* (Berk. & Broome) P.M. Kirk (= *Polyschema bicellulare* Shearer, fide Kirk 1983). The major difference is that *T. australiensis* has smaller conidia (8.5–10 × 4.5–6 µm; Sutton 1989) than *T. uniseptata* (12.5–16 × 6.5–10.5 µm; Kirk 1983). Loose conidia with a similar morphology sometimes found in the air in indoor environments in North America have often been identified as *Endophragmiella*, *Polyschema*, or *Spadicoides* due to lack of conidiophores and the similarity with conidia of *E. uniseptata*, *P. bicellulare*, *S. cordanoides*, and *S. hodgkissii*.

Several attempts to isolate *T. australiensis* from bulk and tape samples failed. The species was found on a tape sample in the USA, but because the sample was

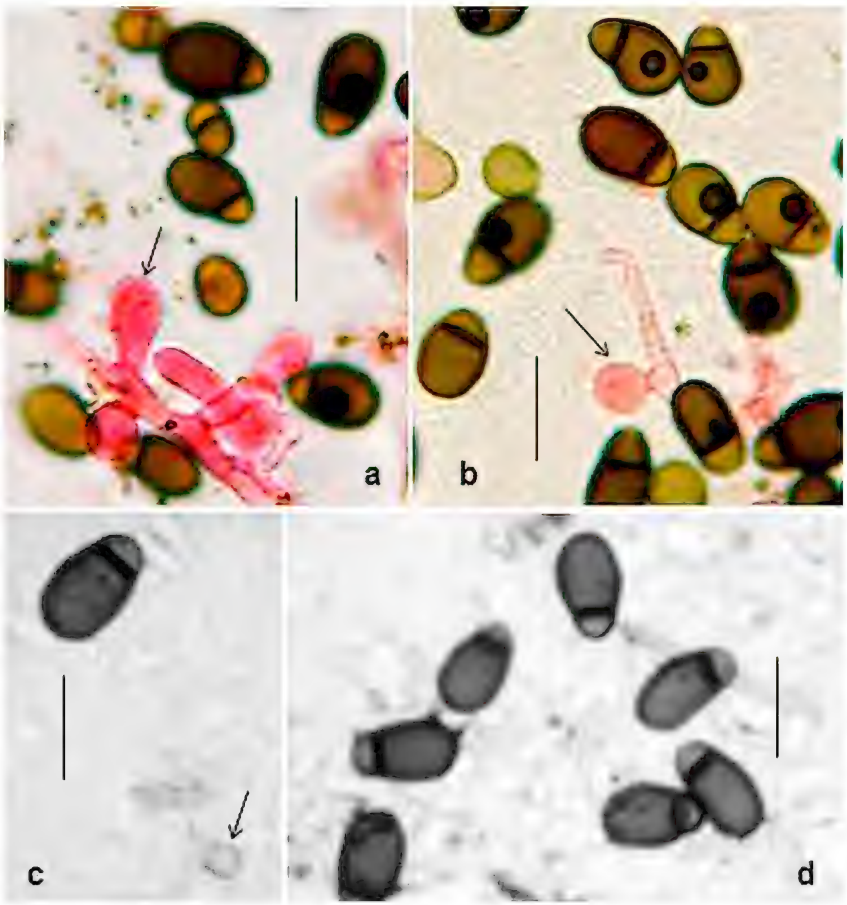


Plate 4. *Triadelphia australiensis* (BPI 884210). a–b. Conidia and conidiogenous cells from a tape sample collected in Canada; c–d. Conidia and conidiogenous cells from a tape sample collected in USA. (Arrows indicate conidiogenous cells.) Scale bars = 10 μm.

consumed during the lab analysis, the excised piece of tape was not retained. Fortunately, although no fungal structures could be found on the tape remnants, photomicrographs were successfully taken from the tape.

Acknowledgments

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Radulidium xigazense sp. nov., *Rhinocladiella tibetensis* sp. nov., and three new records of *Ramichloridium* from China

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Abstract — Two new species, *Radulidium xigazense* and *Rhinocladiella tibetensis,* are described and illustrated from the Qinghai-Tibet Plateau Area, China. *Ramichloridium apiculatum, R. brasilianum,* and *R. musae* represent new records for China. Specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP), and the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

Key words — dematiaceous hyphomycetes, soil fungi, taxonomy

Introduction

During a survey of soil dematiaceous hyphomycetes in China, we found two undescribed species and three new records. The species, all of which produce aseptate conidia on a sympodially proliferating rachis, are described and illustrated from cultures grown on malt extract agar (MEA) (Arzanlou et al. 2007).

Radulidium, established by Arzanlou et al. (2007), is characterized by conidiophores reduced to polyblastic conidiogenous cells that are widest at the base and have straight cylindrical to acicular rachis with crowded pale brown prominent blunt denticles and conidia that are solitary, 0-septate, subhyaline, smooth or verrucose, obovoidal to fusiform, base subtruncate and with a slightly prominent, conspicuously pigmented hilum. Seifert et al. (2011) cite two taxa for the genus.

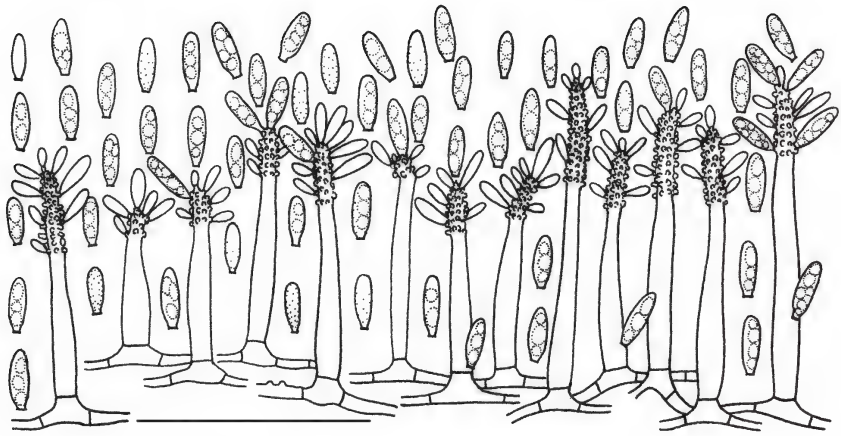

FIG. 1. *Radulidium xigazense* (ex holotype HSAUP II $_{07}$0964).
Conidia, conidiophores, and conidiogenous cells. Scale bar = 25 μm.

Radulidium xigazense Y.M. Wu & T.Y. Zhang, **sp. nov.** FIG. 1

MYCOBANK MB 802277

Differs from *Radulidium subulatum* by its conidia containing several oil droplets and from *R. epichloes* by its smaller conidia.

TYPE: China, Tibet: Xigaze, altitude 3900 m, from a forest soil, 18 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$0964; **isotype** HMAS 196270).

ETYMOLOGY: in reference to the type locality.

COLONIES on MEA effuse, velvety, olivaceous brown. Mycelium mostly immersed, hyphae branched, septate, smooth, subhyaline, 1–2.5 μm wide. CONIDIOPHORES pale brown to subhyaline, mononematous, widest at the base and tapering towards the apex, cylindrical to acicular, generally straight rachis, with crowded, prominent, blunt denticles, smooth, 20–40 μm long, 2 –4 μm wide. CONIDIA solitary, subhyaline, smooth, 0-septate, clavate to fusiform, base subtruncate and with a slightly prominent hilum, containing several oil droplets, 5–7 × 1.5–2 μm.

COMMENTS: Morphologically, *Radulidium xigazense* resembles *R. subulatum* (de Hoog) Arzanlou et al. and *R. epichloes* (Ellis & Dearn.) Arzanlou et al., but *R. epichloes* produces larger (5–11 × 2–3 μm) verruculose conidia, while *R. subulatum* conidia contain no oil droplets (Arzanlou et al. 2007).

Rhinocladiella, established by Nannfeldt (Melin & Nannfeldt 1934), is characterized by conidiophores that are usually branched, brown, and paler towards the apex, intercalary or terminal conidiogenous cells that are cylindrical to acicular, subdenticulate rachis, non-pigmented, and conidia that are solitary,

0-septate, hyaline to subhyaline, smooth, and subglobose to ovoid or long-ellipsoid. Seifert et al. (2011) lists 11 taxa for the genus.

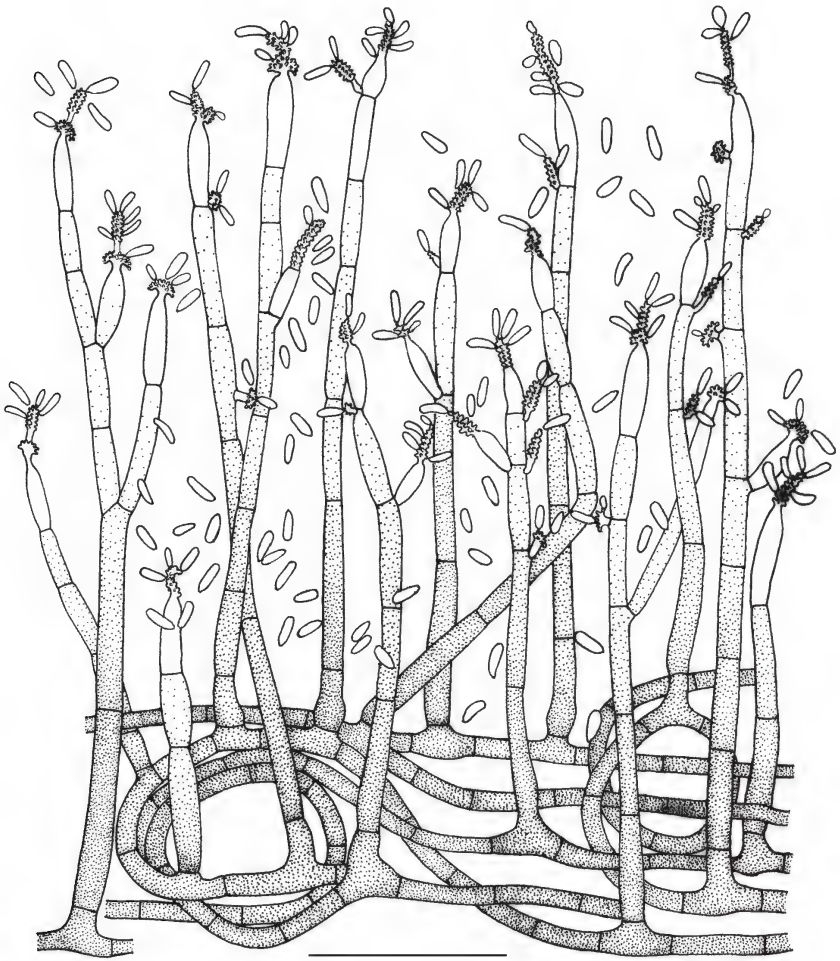

Fig. 2. *Rhinocladiella tibetensis* (ex holotype HSAUP $II_{07}0991$). Conidia, conidiophores, and conidiogenous cells. Scale bar = 25 μm.

Rhinocladiella tibetensis Y.M. Wu & T.Y. Zhang, **sp. nov.** Fig. 2

MycoBank MB 802278

Differs from the *Rhinocladiella* state of *Dictyotrichiella mansonii* by its slightly curved conidia and longer conidiophores, and from *R. pedrosoi* by its narrower and slightly curved conidia.

Type: China, Tibet: Jiangzi, altitude 4000 m, from an everglade soil, 10 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$0991; **isotype** HMAS 196271).

Etymology: in reference to the type locality.

Colonies on MEA effuse, felted, olivaceous to blackish brown. Mycelium superficial or immersed, hyphae branched, septate, smooth, pale brown, 2–3 µm wide. Conidiophores brown, paler towards the apex, macronematous, mononematous, solitary or in groups, erect, septate, smooth, 50–140 µm long and 3–4 µm wide. Conidiogenous cells hyaline, terminal or intercalary, 5–25 µm long and 2–4 µm wide, slightly wider at the middle than the basal part. Conidia hyaline, smooth, 0-septate, clavate, slightly curved, 3–5 × 1–2 µm.

Comments: Morphologically, *Rhinocladiella tibetensis* resembles the *Rhinocladiella* state of *Dictyotrichiella mansonii* Schol-Schwarz and *R. pedrosoi* (Brumpt) Schol-Schwarz, but *D. mansonii* has longer conidiophores and uncurved conidia, and *R. pedrosoi* has wider (3–5 × 2–3 µm) uncurved conidia (Schol-Schwarz 1968).

Ramichloridium, validated by de Hoog (1977), is characterized by straight unbranched brown conidiophores, conidiogenous cells that are integrated, terminal, polyblastic, smooth, golden-brown, subhyaline to pale brown, straight or flexuose, with conspicuous conidiogenous loci or slightly prominent denticles, and conidia that are solitary, 0–1-septate, subhyaline to pale brown, smooth to coarsely verrucose, obovate, obconical to fusiform, and with a prominent hilum. Seifert et al. (2011) lists 21 taxa for the genus.

Ramichloridium apiculatum (J.H. Mill., Giddens & A.A. Foster) de Hoog, Stud. Mycol. 15: 69. 1977. Fig. 3

Colonies on MEA effuse, velvety, olivaceous green. Mycelium superficial or immersed, hyphae branched, septate, smooth, pale to mid brown, 2–3 µm wide. Conidiophores straight, unbranched, smooth or slightly rough, dark brown, up to 100 µm long. Conidiogenous cells integrated, terminal, golden-brown, straight, cylindrical, with conspicuous conidiogenous loci, 25–40 × 2–3.5 µm. Conidia solitary, pale brown, finely verrucose, 0-septate, obovate to obconical, 5–7 × 3–4 µm, hilum conspicuous.

Specimen examined: **CHINA. Tibet**: Xigaze, altitude 3900 m, from a forest soil, 9 Sept. 2007, Y.M. Wu (HSAUP II $_{07}$1427, HMAS 196272).

Ramichloridium apiculatum is reported for the first time from China. Our specimens share the obovate to obconical 0-septate finely verrucose pale brown conidia with a conspicuous hilum and that measure 5–7 × 3–4 µm as described by de Hoog (1977). The species is most similar in conidial shape to *R. cerophilum* (Tubaki) de Hoog, which differs in its smooth conidia with 1–3 short secondary conidia (de Hoog 1977).

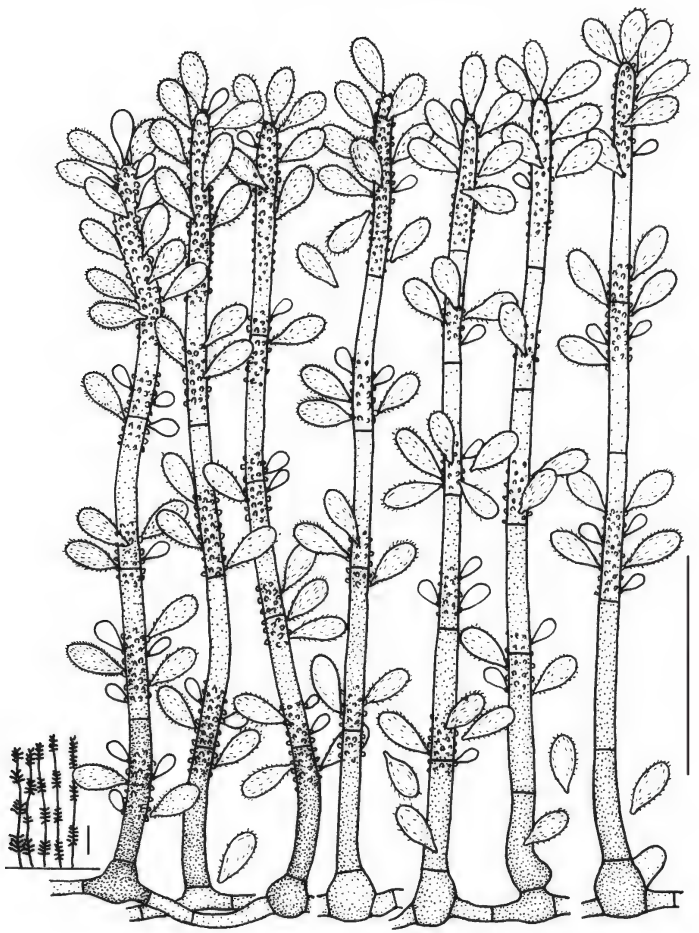

FIG. 3. *Ramichloridium apiculatum* (HSAUP II $_{07}$1427).
Conidia, conidiophores, and conidiogenous cells. Scale bars = 25 μm.

Ramichloridium brasilianum Arzanlou & Crous, Stud. Mycol. 58: 72. 2007. FIG. 4

COLONIES on MEA effuse, velvety to hairy, dark olivaceous-grey. Mycelium superficial or immersed, hyphae branched, septate, smooth or slightly rough, olivaceous-brown, 1.5–2 µm wide. CONIDIOPHORES straight, unbranched, smooth or slightly rough, dark brown, up to 70 µm long. CONIDIOGENOUS CELLS integrated, terminal, subhyaline, straight, cylindrical, with conspicuous conidiogenous loci, 15–30 × 2–3.5 µm. CONIDIA solitary, pale brown, finely verrucose, 0-septate, obovoid to fusiform, 4–6 × 2–2.5 µm, hilum conspicuous.

SPECIMEN EXAMINED: **CHINA. TIBET**: Nagri, altitude 4700 m, from a mountain soil, 18 Sept. 2007, Y.M. Wu (HSAUP II $_{07}$1388, HMAS 196273).

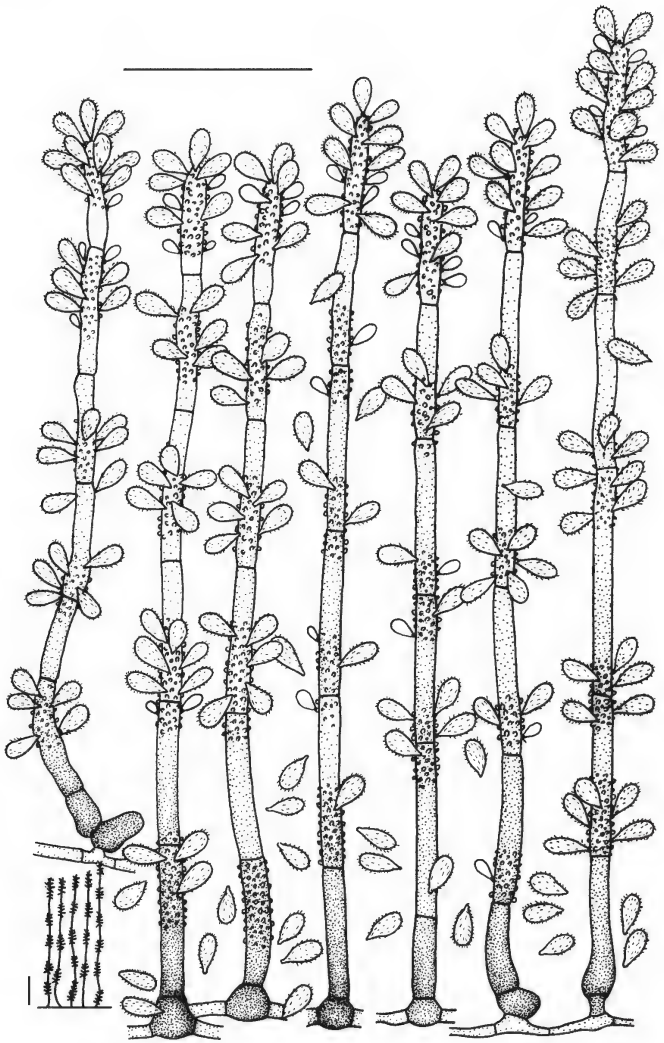

FIG. 4. *Ramichloridium brasilianum* (HSAUP II_{07}1388).
Conidia, conidiophores, and conidiogenous cells. Scale bars = 25 μm.

Ramichloridium brasilianum is also reported for the first time from China. Our specimens share the pale brown conidia that are obovoid to fusiform, finely verrucose, 0-septate, hilum conspicuous, and measure 4–6 × 2–2.5 μm as described by Arzanlou et al. (2007). The species is most similar in conidial shape to *R. biverticillatum* Arzanlou & Crous, which differs in its smooth and smaller (3–4 × 1.5–2.5 μm) conidia (Arzanlou et al. 2007).

Ramichloridium musae (Stahel ex M.B. Ellis) de Hoog, Stud. Mycol. 15: 62. 1977. FIG. 5

COLONIES on MEA effuse, velvety to hairy, olivaceous-grey. Mycelium superficial or immersed, hyphae branched, septate, smooth, olivaceous- brown, 1–2 µm wide. CONIDIOPHORES straight, unbranched, smooth or slightly rough, brown, up to 160 µm long. CONIDIOGENOUS CELLS integrated, terminal, subhyaline, straight, cylindrical, with conspicuous conidiogenous loci, 20–30 ×

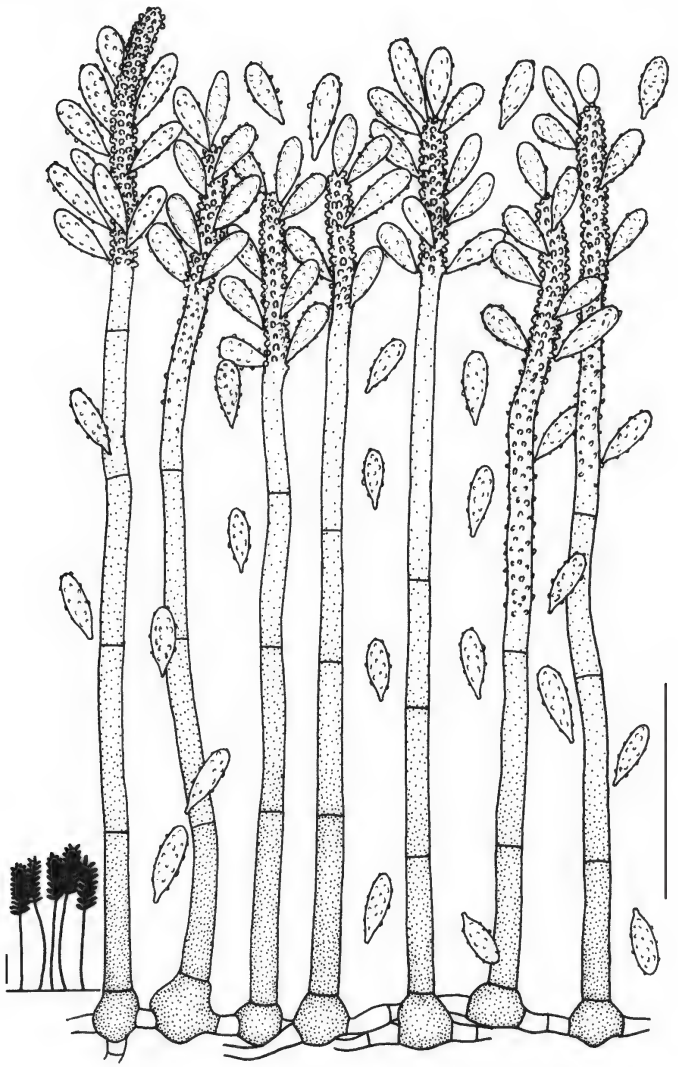

FIG. 5. *Ramichloridium musae* (HSAUP II $_{07}$0576).
Conidia, conidiophores, and conidiogenous cells. Scale bars = 25 µm.

2–2.5 µm. Conidia solitary, pale brown, finely verrucose, 0-septate, obovate to obconical, 7–9 × 2–3 µm, hilum conspicuous.

Specimen examined: **CHINA. Tibet**: Shannan, altitude 3500 m, from a grassland soil, 10 Jun. 2007, Y.M. Wu (HSAUP II$_{07}$0576, HMAS 196274).

Ramichloridium musae is reported for the first time from China. Our specimens share the pale brown conidia that are obovate to obconical, finely verrucose, 0-septate, hilum conspicuous, and measure 7–9 × 2–3 µm as described by de Hoog (1977). The species is most similar in conidial shape to *R. cerophilum*, which is distinguished by smooth and smaller conidia (6–7 × 2–3 µm; de Hoog 1977).

Acknowledgments

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A new species of *Emericella* from Tibet, China

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Abstract — *Emericella miraensis* sp. nov. is described and illustrated. It was isolated from the alpine plant *Polygonum macrophyllum* var. *stenophyllum* from Tibet, China, and is characterized by ascospores with star-shaped equatorial crests. The new species is distinguished from other *Emericella* species with stellate ascospores (e.g., *E. variecolor, E. astellata*) by its violet ascospores and verrucose spore ornamentation. ITS and β-tubulin sequence analyses also support *E. miraensis* as a new species.

Key words —*Aspergillus*, endophytic fungi, phylogeny, taxonomy

Introduction

Berkeley (1857) established *Emericella,* a teleomorph genus associated with *Aspergillus,* for the type species *E. variecolor* Berk. & Broome (Geiser 2009, Peterson 2012). To date, 36 species have been described and recorded worldwide (Kirk et al. 2008). *Emericella* species are usually isolated from soil (Samson & Mouchacca 1974, Horie et al. 1989, 1990, 1996, 1998, 2000; Stchigel & Guarro 1997) but sometimes also from stored foods, herbal drugs, and grains or occasionally from hypersaline water (Zalar et al. 2008) or living plants (Berbee 2001, Thongkantha et al. 2008, Zhang et al. 2011).

During a survey of endophytic fungi associated with *Polygonum macrophyllum* var. *stenophyllum* (Meisn.) A.J. Li in Tibet, China, an unidentified endophytic fungus was isolated from the roots of the alpine medicinal plant. Our morphological and molecular data confirm the fungus as a new *Emericella* species.

Materials & methods

Plant samples of *P. macrophyllum* var. *stenophyllum* were collected at 4850 m altitude from the mountain steppe of Mira Hill at Nyingchi County, in Tibet, China, in June

2010. Plant samples and their rhizosphere soils were collected and kept in plastic bags together for transporting to the laboratory.

For fungal isolation, three pieces of healthy roots of individual plants were randomly selected and removed from each of the five *P. macrophyllum* var. *stenophyllum* plants, rinsed under tap water to remove soil and litter, and washed in sterile de-ionized water. Each root was cut into 20 mm long segments, and the surfaces were sterilized in 75% ethanol for 1 min, 3% NaClO for 3 min, and 75% ethanol for 30 s, and then rinsed in sterilized water three times. These root segments were cut into approximately 1 mm long portions and inoculated on potato dextrose agar (PDA) in 90 mm diam. Petri dishes. The cultures were kept in a dark incubator at 25°C to obtain their anamorphic stages. The fungal mycelium was sub-cultured in Czapek yeast extract agar (CYA), PDA, and malt extract agar (MEA) for morphological observations.

The fungal isolates were identified morphologically from colony characters (e.g., colour, growth rate, hyphae, and margin characters) and micro-characters (conidia and ascospores) on CYA and PDA medium. Strains were incubated at 25°C for 14 d, after which colonies and mycelia were observed using a light microscope (Zeiss Axio ImagerA1) and scanning electronic microscope (SEM; JSM-6510LV, Japan). Cultures have been conserved in the Chinese General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (CGMCC).

DNA was extracted from the examined strain using the E.Z.N.A.™ Fungal DNA Mini Kit (Omegabiotek, Norcross, USA) according to the manufacturer's protocol. The internal transcribed spacer (ITS) region was amplified with primer pair ITS1/ITS4 (White et al. 1990) and the β-tubulin gene with primer pair T1/T22 (O'Donnell & Cigelnik 1997) according to Zhang et al. (2012). PCR products were examined in 1% agarose gels (mixed with goldview) by electrophoresis visualized under UV light. After purification with mini-columns (Sangon, China), purified DNA was directly sequenced in the ABI Prism 377 DNA sequencer (Applied Biosystems, USA).

Similar sequences were retrieved from GenBank using the NCBI Blast program (Altschul et al. 1997), most previously published in Peterson (2008) and Zalar et al. (2008). *Aspergillus karnatakaensis* was chosen as outgroup based on Peterson's (2008) analyses. Sequences were aligned with Clustal X 1.81. Neighbor joining (NJ) trees were constructed with the Kimura two-parameter model (Kimura 1980) and maximum parsimony (MP) trees with the close-neighbor-interchange (CNI) search method on random trees on Mega version 5.0 (Tamura et al. 2011).

Results

Taxonomy

Emericella miraensis L.C. Zhang, Juan Chen & S.X. Guo, **sp. nov.** Fig. 1

MycoBank MB 800444

Differs from *Emericella stella-maris* and *E. variecolor* by its violet ascospores with verrucose convex walls.

Type: China, Tibet, Nyingchi County, Mira Hill, 29°53′N 92°50′E, 4850 m, from roots of *Polygonum macrophyllum* var. *stenophyllum*, June 2010, Li-Chun Zhang (**Holotype**, CGMCC3.14984; GenBank, JQ268604, KC342577).

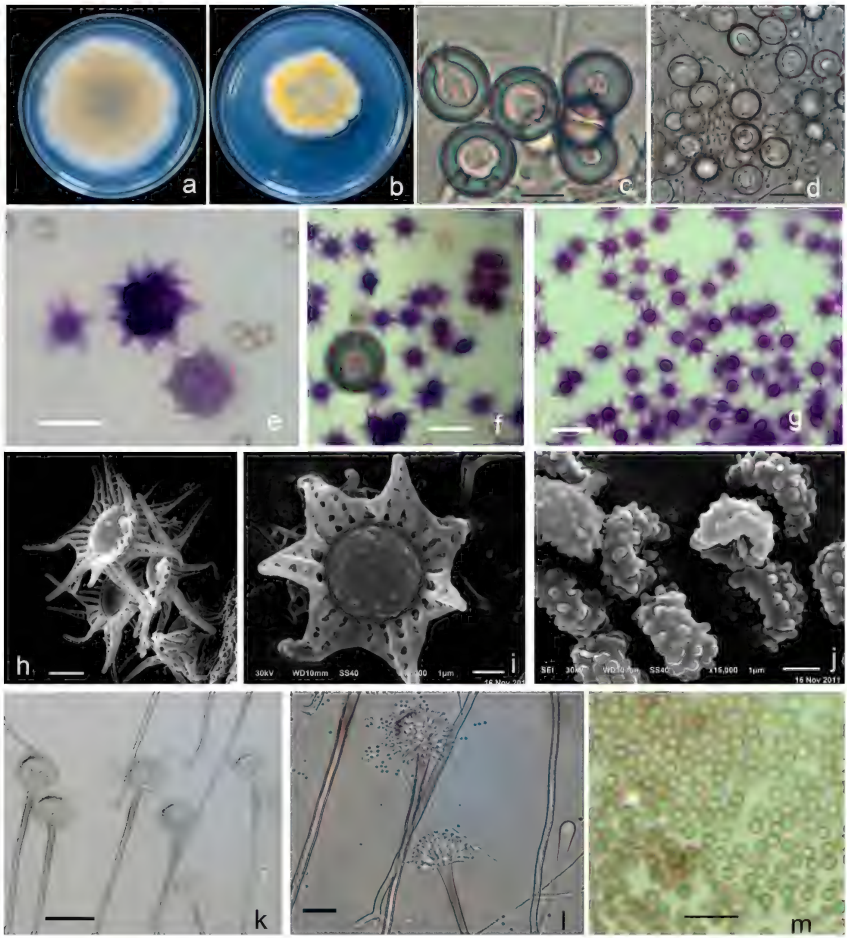

FIGURE 1. *Emericella miraensis* (holotype). Morphological characters. **a**. Colonies on PDA after 2 week at 25°C; **b**. Colonies on CYA after 2 week at 25°C; **c**, **d**. Hülle cells; **e**. Asci in different developmental stages views; **f**. Hülle cells, asci and ascospores; **g**. Stellate ascospores; **h**, **i**. Scanning electron micrograph of ascospores showing the side views and verrucose ornamentation on the convex surface; **j**, Scanning electron micrograph of conidia; **k**, **l**. Conidiophores; **m**. Conidia. Bars: c, e–g, m = 10 μm; d, l = 20 μm; h = 2 μm; i, j = 1 μm; k = 100 μm.

ETYMOLOGY — *miraensis* refers to the type locality, Mira Hill.

COLONIES on PDA spreading broadly, attaining 7.2–7.8 cm diam. in 14 days at 25°C. Aerial mycelium khaki, marginal mycelium white, irregular, and small amounts exudates droplets and granular produced in center after two weeks, reverse pale yellowish white, and then becoming gray purple-brown. Ascomata

abundantly produced, gray green, subglobose, with numerous, globose to ovoid shaped Hülle cells, 15–20 mm diam.

Asci 8-spored, globose, 10 × 20 μm, firstly pink-purple, becoming violet in mature. Ascospores violet, with 6–7 μm denotative crests stellate in surface view, 6.0–10.0 μm (including stellate tentacles); spore bodies subglobose, verrucose on the surface, 4.0–5.0 μm diam., convex-lens form, with two stellate, broad equatorial crests in side view; undissected part of crests ≤ 1 μm, every angle of stellate crests regularly ornamented with three longitudinal striations, transverse striation arranged irregularly between two longitudinal striations.

Anamorphic stage: Conidial heads grayish olive, radiate to short columnar. Conidiophores abundant, columnar, straightforward, stipes smooth, brownish and nonseptate, generally 275–345 μm long × 8.5–13.0 μm wide, dark colour near the conidia; vesicles abundant, radiate, subclavate to flask-shaped, hyaline to pale brownish green, 2.0–3.5 μm wide × 9.0–17.0 μm long, covered in the upper half by metulae, which are hyaline to pale brown, 15.5–18.5 × 9.0–10.0 μm; phialides flask-shaped, hyaline, 10–16.5 × 3.5–5.5 μm.

Conidia globose to subglobose, verrucose, appearing grayish green in mass, diameter 1.5–4.0 μm, strawberry appearance in SEM. Colonies on MEA spreading broadly, attaining a diameter of 5.3–5.7 cm in 14 days at 25°C; appearance same as on PDA, but ascomata arranged in concentric circles, particularly on colony margins, grayish green, edge mycelium white. Colonies on CYA spreading slowly, attaining 4.0–4.5 cm diam. after 14 d at 25°C, bright yellow, white edge, consisting of a thin mycelial felt, slightly granular in colony center due to formation of Hülle cells (thick-walled refractive cells like chlamydospores) and conidia abundant, reverse yellowish brown, edge mycelium white.

Phylogenetic analysis

The sequences for our new taxon have been deposited in GenBank. The ITS data set consisted of 28 sequences and 550 characters, of which 98 are parsimony informative sites. MP analysis produced 97 trees (TL = 139, CI = 0.813, RI = 0.834), of which one is shown in Fig. 2. NJ analysis (not shown) created similar topology to MP analysis. All *Emericella* sequences analyzed were divided into two large clades and four subclades. *Emericella miraensis* formed a well-supported clade (Clade II; BP = 100%) with other related species, such as *E. stella-maris, E. astellata, E. variecolor, E. qinqixianii, E. appendiculata,* and *E. filifera*. These taxa all have star-shaped ascospores, but are distinguished from one another by color or ascospore ornamentation (Table 1).

The β-tubulin data set contained 19 sequences and 420 characters, of which 84 were parsimony informative sites. MP analysis produced 9 trees

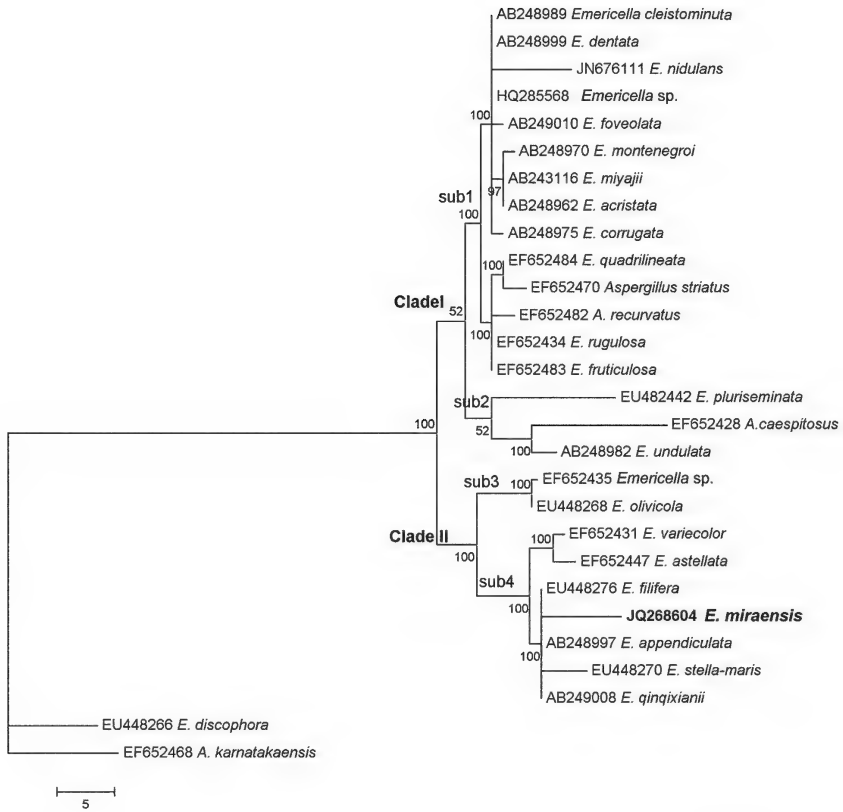

FIGURE 2. One of the MP trees obtained based on phylogenetic analysis of ITS sequence data of *Emericella/Aspergillus*. Numbers above branches are bootstrap values ≥ 50%.

(TL = 172, CI = 0.784, RI = 0.804; one tree is shown in FIG. 3). MP analysis of β-tubulin produced topologies very similar to those obtained by ITS analysis. All *Emericella* sequences analyzed were divided into three large clades, with *E. miraensis* forming a well-supported clade (BP = 100%) with three other species (*E. stella-maris, E. astellata, E. variecolor*).

Discussion

The *Emericella miraensis* specimens were isolated from the roots of an alpine plant. Microscopic examination showed ascomata embedded in masses of Hülle cells and violet ascospores with two stellate equatorial crests. In addition, their anamorphic *Aspergillus* stage also was observed on CYA,

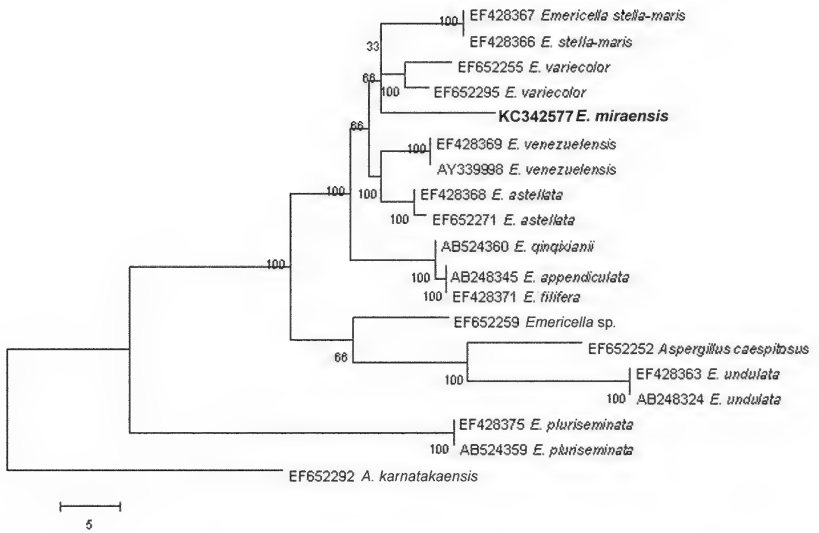

FIGURE 3. One of the MP trees obtained based on phylogenetic analysis of β-tubulin sequence data from *Emericella/Aspergillus*. Numbers above branches are bootstrap values ≥ 50%.

PDA, and MEA. These are some typical morphological features associated with *Emericella* (Malloch & Cain 1972). *Emericella miraensis* differs from the closely related *E. stella-maris* and *E. variecolor* in having ascospores with tuberculate or verrucose convex walls and of a different color (TABLE 1). In previous studies, the six *Emericella* species having ascospores ornamented in a star-shape pattern included *E. variecolor* (Berkeley 1857), *E. astellata* (Horie 1980), *E. pluriseminata* (Stchigel & Guarro 1997), *E. venezuelensis* (Frisvad & Samson 2004), *E. stella-maris*, and *E. olivicola* (Zalar et al. 2008). *Emericella pluriseminata* did not produce aspergilla on any media (Frisvad & Samson 2004, Zalar et al. 2008), while *E. miraensis* forms conidiophores and conidia on three conventional media: CYA, PDA, and MEA. Although *E. miraensis* resembles *E. variecolor* in ascospore colour, its ascospores are obviously verrucose as seen in SEM, while *E. variecolor* ascospores have smooth convex walls. The phylogenetic trees (FIGS 2–3) indicate a close relationship between *E. miraensis* and *E. stella-maris,* which differs in its orange-red ascospore color. *Emericella miraensis* also can easily be distinguished from *E. venezuelensis*, which has violet brown ascospores with triangular flaps on their convex sides. In addition, the nonseptate conidiophores of *E. miraensis* distinguish it from *E. stella-maris* and *E. astellata*, whose conidiophores are always septate. Ascospore morphology is the most important diagnostic character for *Emericella* species (Horie 1980).

Table 1. Ascospore characters of *Emericella miraensis* and related species.

Species	Ascospores		
	Ascospore shape	Color	Surface ornamentation
E. variecolor	stellate	violet	smooth
E. stella-maris	stellate	orange-red	smooth
E. astellata	stellate	reddish purple–reddish brown	smooth
E. qinqixianii	lenticular	violet-brown	smooth
E. appendiculata	lenticular	violet-brown	capitate appendages
E. filifera	subglobose	brownish red	capitate appendages
E. venezuelensis	stellate	violet brown	triangular flaps
E. miraensis	stellate	violet	verrucose

Most *Emericella* species are more adapted to dry warm climates than to humid cold environments (Samson & Mouchacca 1974, Zalar et al. 2008). However the host of *E. miraensis*, *P. macrophyllum* var. *stenophyllum,* is an alpine plant that occurs in mountain steppes up to 4850 m, with annual precipitation of 443.6 mm (Luo et al. 2003).

Acknowledgments

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Jayarambhatia rhizophorae gen. et sp. nov., an asexually reproducing fungus from Goa, India

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Abstract — A new hyphomycete genus, *Jayarambhatia,* is proposed, characterized by mononematous branched septate pale brown smooth conidiophores with terminal hyaline phialidic conidiogenous cells with long slender necks and minute collarettes. Numerous smooth aseptate narrowly obclavate filiform conidia double over and pack densely together to form ovate mucous heads on the tips of the conidiogenous cells. The type species was collected from decomposing litter of *Rhizophora mucronata* in an intertidal mangrove area in Goa, India.

Key words — systematics, taxonomy

Introduction

Unique organisms inhabit mangroves in the intertidal saline zones. This highly productive ecosystem supports many marine organisms including fish (Thatoi & Biswal 2008). Fungi inhabiting this ecosystem are called manglicolous fungi, and they degrade mangrove litter and other detritus (Nambiar & Raveendran 2009). The entire coast of Goa with its backwaters and seven major rivers is lined with diverse mangrove vegetation.

During a survey of manglicolous fungi of Goa, a unique asexually reproducing fungus was encountered on decomposing twigs and a viviparous seedling of *Rhizophora mucronata* Lam. (*Rhizophoraceae*). The fungus produced mononematous branched septate pale brown smooth conidiophores terminating in phialidic conidiogenous cells with long slender necks and smooth filiform conidia that double over and pack closely together to form mucous heads at the tips of the conidiogenous cells. These unique features warrant accommodation of the fungus in a new genus. The monotypic genus is compared with four closely related genera (*Atrosetaphiale, Kmetiopsis, Natarajania, Phaeohiratsukaea*) and *Dictyochaeta lilliputiana, D. minutissima,* and *D. uncinata,* and its sole species is described and illustrated.

Materials & methods

Freshly gathered mangrove litter from intertidal area was brought to the laboratory in polythene bags, incubated for 2–3 days and examined under a stereomicroscope. The fungus was carefully picked up with a sterile needle and mounted in either lactophenol or lactophenol cotton blue mountant. The slides were observed under a light microscope. The holotype is conserved in the herbarium of the Indian Agricultural Research Institute, New Delhi, India (HCIO).

Taxonomy

Jayarambhatia Pratibha, **gen. nov.**

MycoBank MB803753

Differs from *Atrosetaphiale, Kmetiopsis, Natarajania, Phaeohiratsukaea,* and *Dictyochaeta* by solitary branched conidiophores lacking setae, terminal monophialidic conidiogenous cells with elongated slender necks terminating in minute collarettes with filiform conidia that are doubled over and packed closely together in mucous heads.

Type species: *Jayarambhatia rhizophorae* Pratibha

Etymology: In honor of Prof. D. Jayarama Bhat, Department of Botany, Goa University, Goa, India.

Colonies effuse, white, velvety. Mycelium partly immersed in the substrate. Conidiophores mononematous, macronematous, erect, straight, solitary, sometimes branched, smooth, brown. Conidiogenous cells monophialidic, terminal, integrated, cylindrical, terminating with a narrow, elongated, slender neck. Conidial head ovate, mucous, within which numerous conidia are doubled over and densely packed. Conidia smooth, hyaline, aseptate, filiform, narrowly obclavate, straight to flexuous when mounted in water.

Jayarambhatia rhizophorae Pratibha, **sp. nov.** Figs 1, 2

MycoBank MB803754

Differs from species of *Atrosetaphiale, Kmetiopsis, Natarajania, Phaeohiratsukaea*, and *Dictyochaeta* by monophialidic terminal conidiogenous cells with elongated slender necks terminating with a minute collarette, and filiform conidia irregularly doubled over within a mucous head.

Type: India, Goa, Chorao, on decomposing litter of *Rhizophora mucronata*, 8 January 2013, coll. J. Pratibha (**Holotype,** HCIO 51502).

Etymology: Referring to the host genus.

Colonies effuse, white, velvety. Mycelium light brown, smooth, septate, 2–3 µm wide, partly immersed in the substrate. Conidiophores mononematous, macronematous, erect, straight to flexuous, solitary, septate, rarely branched at the base, smooth, pale brown, 75–150 × 4.5–7 µm. Conidiogenous cells monophialidic, terminal, integrated, cylindrical, smooth, 15–20 × 5–6 µm, with an elongated, narrow, slender neck terminating in a small collarette. Conidial head ovate, mucous, 15–20 µm long, within which numerous conidia are

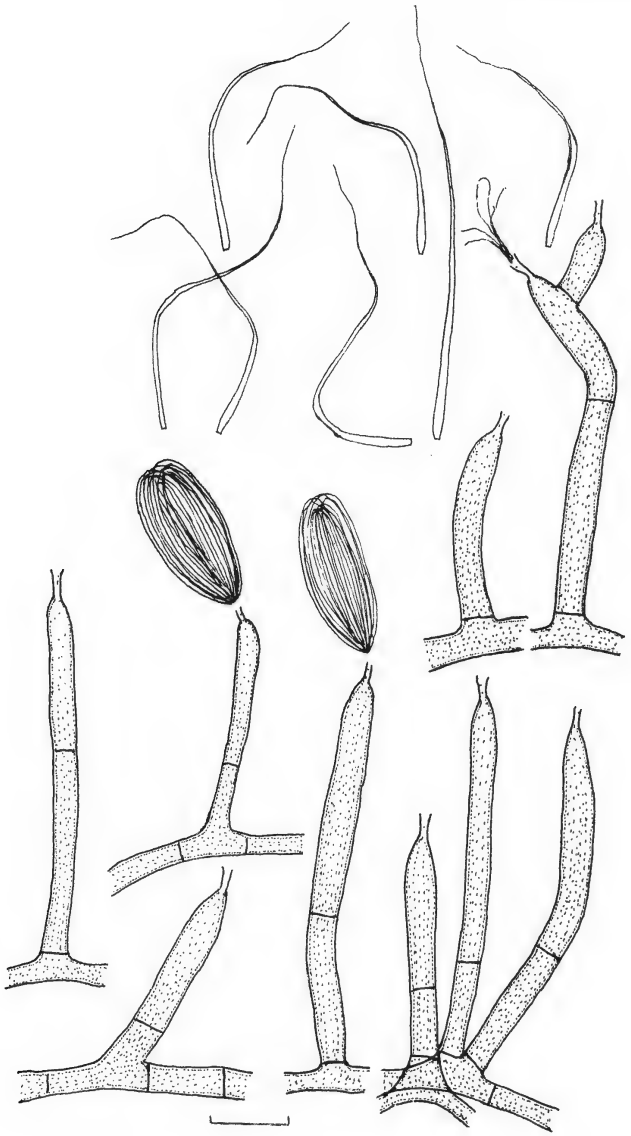

FIG.1. *Jayarambhatia rhizophorae* (holotype, HCIO 51502): Conidiophores, conidiogenous cells, and conidia. Scale bars = 10 μm.

doubled over and densely packed. CONIDIA smooth, hyaline, aseptate, filiform, narrowly obclavate, straight to flexuous, 30–50 × 1 μm when mounted in water.

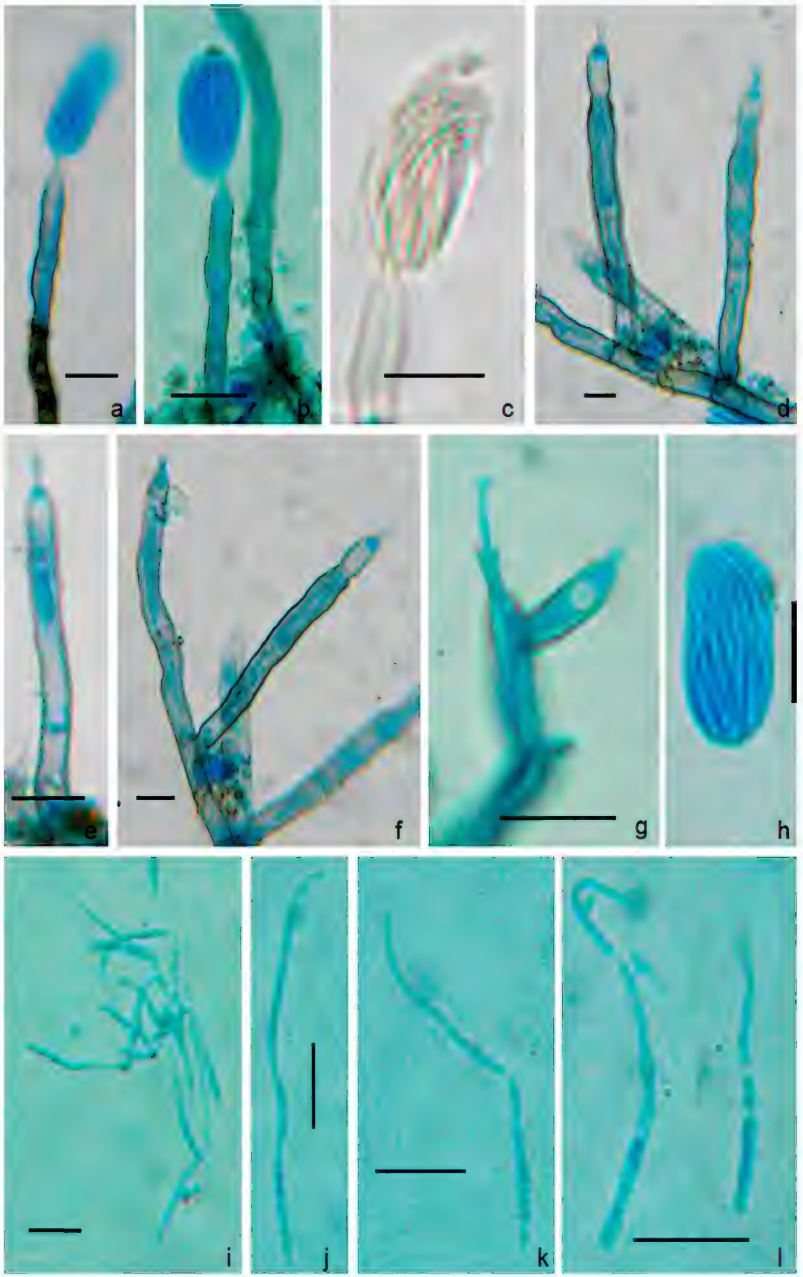

Fig. 2. *Jayarambhatia rhizophorae* (holotype, HCIO 51502): a–f. Conidiophores; g. Conidiogenous cells; h. Mucous conidial head; i–l. Conidia. Scale bars = 10 µm

Discussion

Jayarambhatia is morphologically similar to anamorphic genera such as *Atrosetaphiale* Matsush., *Kmetiopsis* Bat. & Peres, *Natarajania* Pratibha & Bhat, *Phaeohiratsukaea* Udagawa & Iwatsu, and three species of *Dictyochaeta* — *D. lilliputiana* R.F. Castañeda, *D. minutissima* A. Hern.-Gut. & J. Mena, and *D. uncinata* R.F. Castañeda & W.B. Kendr. (Table 1). *Atrosetaphiale, Kmetiopsis,* and the three *Dictyochaeta* spp. are similar to *Jayarambhatia* in producing a mucous head of filiform or acerose conidia on monophialidic conidiogenous cells (Batista & Peres 1960, Hernandez-Gutiérrez & Mena Portales 1996, Batista & Peres 1960, Castañeda 1986, Castañeda et al. 1998, Matsushima 1995). *Natarajania* and *Phaeohiratsukaea* produce phialidic conidiogenous cells with an elongated, slender neck with a collarette (Pratibha & Bhat 2005, Udagawa & Iwatsu 1990). None of these fungi have filiform conidia doubled over within mucous heads. *Atrosetaphiale* produces discrete, ampuliform, numerous conidiogenous cells. In *Dictyochaeta lilliputiana* conidiophores are ampulliform and conidiogenous cells are without neck and conspicuous

Table 1. Comparison between *Jayarambhatia* and related genera and species

Genera	Habitat	Conidiophores	Conidiogenous cells	Conidia
Atrosetaphiale	Terrestrial litter	Mononematous, solitary, unbranched	Monophialidic, discrete, numerous, ampuliform	Filiform, slimy, long, flexuous, hyaline
Dictyochaeta lilliputiana	Terrestrial litter	Mononematous, solitary, unbranched, aseptate, ampuliform	Monophialidic, without neck and conspicuous collarette	Filiform, slimy, curved, hyaline
Dictyochaeta minutissima	Terrestrial litter	Mononematous, solitary, branched	Monophialidic, with 1–3 percurrent proliferations	Acerose to fusiform, slightly curved, hyaline to sub-hyaline
Dictyochaeta uncinata	Terrestrial litter	Mononematous, solitary, unbranched, setose.	Monophialidic with conspicuous narrow collarette	Filiform, slimy, long, flexuous, hyaline
Kmetiopsis	Terrestrial litter	Sporodochial with pseudostroma	Monophialidic, without neck and collarette	Solitary, slimy, falcate, hyaline, ends pointed
Natarajania	Terrestrial litter	Mononematous, fasciculate, verrucose towards apex	Monophialidic, with long, cylindrical neck and collarette	Solitary, slimy, ellipsoidal, dark brown
Phaeohiratsukaea	Terrestrial litter	Mononematous, fasciculate, branched	Mono- to polyphialidic, with short neck and collarette	Catenate, dry, ellipsoidal, brown with germ slit
Jayarambhatia	Mangrove litter	Mononematous, solitary, branched	Monophialidic with slender neck and minute collarette	Filiform, slimy, long, flexuous, hyaline, tightly doubled over, forming mucous head

collarette. *Dictyochaeta minutissima* produces monophialidic conidiogenous cells with 1–3 percurrent proliferations and acerose to fusiform conidia. In *Dictyochaeta uncinata* conidiophores are unbranched, it produces setae, and the conidiogenous cells have a conspicuous narrow collarette. In *Kmetiopsis* the conidiophores are sporodochial with a pseudostroma and the conidia are acerose. In *Natarajania* the conidiophores are unbranched and verrucose at the upper part and the conidia are ellipsoidal and dark brown. In *Phaeohiratsukaea* the conidiophores are percurrent, conidiogenous cells are mono- to polyphialidic and the conidia are ellipsoidal, brown with a germ slit and formed in chains. The combination of diagnostic characters found in *Jayarambhatia* is not shown by any other genus of phialidic fungi. Moreover, all of the fungi discussed above were isolated from terrestrial litter, whereas *Jayarambhatia* was isolated from mangrove litter from intertidal region.

Acknowledgments

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Sympodioplanus goaensis sp. nov. from Goa, India

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Abstract — A new species, *Sympodioplanus goaensis*, occurring on decaying unidentified twigs collected from Goa, India, is described and illustrated. The fungus is characterized by numerous conidiogenous cells that are polyblastic, sympodial, integrated, and terminal as well as intercalary and by naviculiform 1–2-septate conidia

Key words — taxonomy, litter fungi

Introduction

During a biodiversity survey of litter-degrading microfungi from forests of Western Ghats in Goa, an undescribed anamorphic fungus with morphological characteristics of *Sympodioplanus* R.C. Sinclair & Boshoff was found on fallen decomposing twigs of an unidentified plant. The fungus is described, illustrated, and compared with other *Sympodioplanus* spp.

Materials & methods

Freshly gathered litter samples from Sonal forests of Goa were taken to the laboratory in polythene bags, incubated for 2–3 days, and examined under a stereomicroscope. Fungal fruiting bodies were carefully picked up with a sterile needle, mounted in lactophenol, and observed under a light microscope. Several fungi were observed, one of them an undescribed species of *Sympodioplanus*, which is described here. The holotype is conserved in the herbarium of the Indian Agricultural Research Institute, New Delhi, India (HCIO).

Taxonomy

Sympodioplanus goaensis Pratibha, **sp. nov.** (Figs 1, 2)

MycoBank MB804078

Differs from *Sympodioplanus capensis* by its larger conidia with fewer septa and from *S. yunnanensis* by its smaller conidia with fewer septa.

Type: India, Goa, Sattari, Sonal, tropical forests of Western Ghats, on decomposing litter of unidentified tree, 26 January 2013, coll. J. Pratibha (**Holotype** HCIO 51503).

Etymology: In reference to the biodiversity rich Goa State.

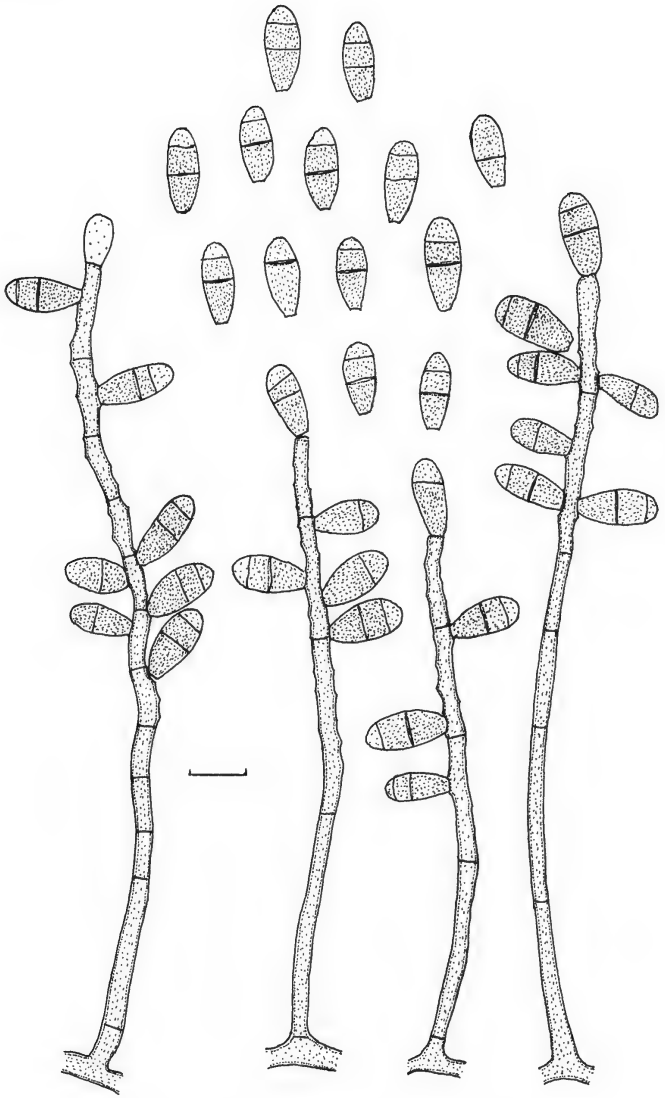

Fig. 1. *Sympodioplanus goaensis* (holotype HCIO 51503): Conidiophores, conidiogenous cells, and conidia. Scale bar = 20 µm.

Colonies on natural substrate effuse, hairy, dark brown. Mycelium partly superficial, partly immersed in the host tissue, composed of light brown, smooth, branched, thin-walled, 1.5–3 µm wide hyphae. Conidiophores macronematous, mononematous, erect, straight to flexuous, smooth, thick-

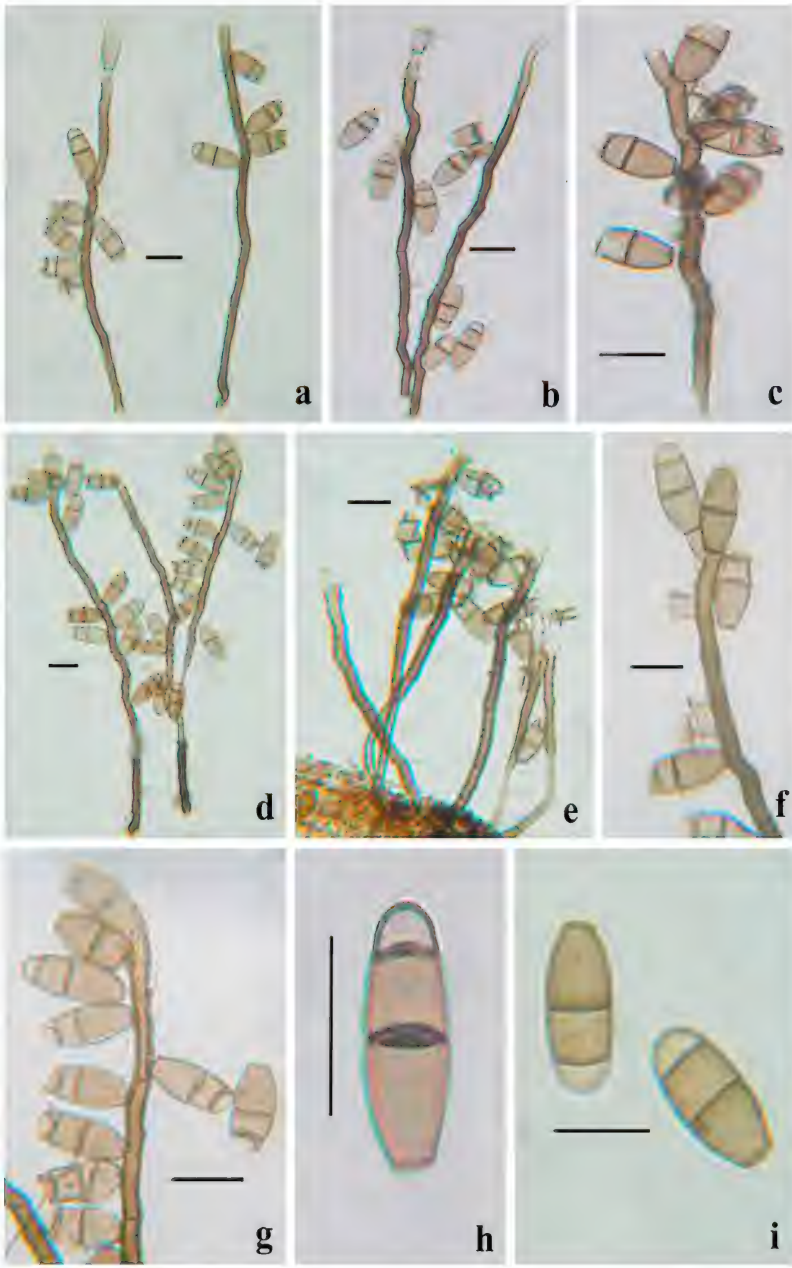

Fig. 2. *Sympodioplanus goaensis* (holotype HCIO 51503): a–g. Conidiophores, conidiogenous cells, and conidia; h–i. Conidia. Scale bars = 20 µm.

walled, dark brown, paler towards the apex, unbranched, 160–285 × 4–6.5 µm. Conidiogenous cells polyblastic, terminal as well as intercalary, sympodial, thick-walled, 8–10 × 2–3 µm, bearing flat scars. Conidia solitary, dry, light brown, thin-walled, smooth, naviculiform, rounded at the apex, truncate at the base; basal cells light brown, apical cell subhyaline, 1–2-septate, 22–36 × 8–12 µm.

Discussion

The genus *Sympodioplanus* is typified by *S. capensis* R.C. Sinclair & Boshoff, which was collected from dead, decorticated wood in South Africa (Sinclair et al. 1997). The genus is mainly characterised by conidiophores with sympodial proliferation with numerous conidiogenous loci and naviculiform, euseptate conidia. Yang et al. (2012) added a second species, *S. yunnanensis* G.Z. Yang & Z.F. Yu from China on submerged leaves of an unidentified dicotyledonous plant. These two articles comprise the total published knowledge of *Sympodioplanus*. *Sympodioplanus goaensis* differs from *S. capensis* and *S. yunnanensis* by length of the conidiophores and septation and size of the conidia (Table 1). All three species have polyblastic sympodial conidiogenous cells.

Table 1: Comparison of *Sympodioplanus* species

Species	Conidiophores	Conidia
S. capensis	Macronematous, moderately geniculate, <65 µm long	Naviculiform, 3(–5)-septate, 13–16 × 2.5–4 µm
S. goaensis	Macronematous, straight, 160–285 µm long	Naviculiform, 1–2-septate, 22–36 × 8–12 µm
S. yunnanensis	Macronematous, straight, 70–205 µm long	Spindle shaped, 4–7-septate, 38–56 × 6–10.8 µm

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Thanks to the University Grants Commission, New Delhi, for post doctoral research grant support. I am indebted to Dr. Eric McKenzie, Landcare Research, New Zealand, and Dr. Xiu-Guo Zhang, Department of Plant Pathology, Shandong Agricultural University, China for kindly reviewing the manuscript for Mycotaxon. I take this opportunity to thank my guide and Head of Department of Botany, Goa University Prof. M. K. Janarthanam, for supporting this work.

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Contributions to the family *Thyridiaceae*. New data on *Sphaeria mutabilis*

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Abstract — Type specimens of the genera *Balzania*, *Mattirolia*, *Thyridium*, and *Thyronectroidea* were studied. We accept *Mattirolia* (five species) and *Thyridium* (four species) in the *Thyridiaceae* and regard *Balzania* and *Thyronectroidea* as synonyms of *Mattirolia*. The occurrence of *Mattirolia roseovirens* in Spain represents only the second collection since its original description. Four new combinations are proposed: *Mattirolia mutabilis*, *M. ohiensis*, *M. platensis*, and *Thyridium lasiacidis*. Lectotypes are designated for *M. mutabilis* and *M. ohiensis*. A key is provided to species in the *Thyridiaceae*.

Key words — *Ascomycota*, *Lasiella mutabilis*, systematics, taxonomy

Introduction

The yellowish color that appears in the fruiting bodies of some *Pyrenomycetes* sensu lato has raised confusion among mycologists throughout history, e.g., Quélet (1875), Saccardo (1883), Winter (1884–87), and Munk (1957). Although the coloration suggests the order *Hypocreales*, detailed microscopic studies indicate that species with yellow ascomata can be very different and phylogenetically distant.

While studying samples on logs and dead branches of *Genista scorpius* (L.) DC. (*Fabaceae*), a number of fungi with yellow ascomata were collected that do not belong in the *Hypocreales*. This scrub tree is common in the green oak forest of Tamajón (Guadalajara, Spain) growing on basic ground limestone. These samples were identified as *Mattirolia roseovirens* representing the family *Thyridiaceae* (*Sordariomycetidae*), although macroscopically they resembled *Pseudotrichia mutabilis* in the *Pleosporomycetidae*.

The study of these samples led us to revise species in *Thyridiaceae* for comparison with other species having yellow ascomata. The *Thyridiaceae*

previously studied by Yue & Eriksson (1987), Eriksson & Yue (1989), Barr (1990), and Eriksson & Hawksworth (1991), currently consist of five genera and 22 species (Kirk et al. 2008), although Lumbsch & Huhndorf (2010) have accepted only four genera. Most of the studied species have been collected only once and are represented only by type specimens, which are generally old and currently of uncertain taxonomic position. In this paper, we provide an account of two genera with nine species accepted in the *Thyridiaceae*.

Materials & methods

Mattirolia roseovirens was collected on *Genista scorpius* during surveys carried out in the spring of 1996 and 2008 at Tamajón (Guadalajara, Spain). Specimens are deposited in AH and compared with other *M. roseovirens* specimens, including the type material from BPI, FH, and S. These collections were compared with other species of the *Thyridiaceae* from the herbaria B, K, LPS, NY, UME, including type specimens of *Balzania platensis*, *Sinosphaeria lasiacidis*, *Teichospora ohiensis*, *Thyronectria chrysogramma*, and *Thyridium flavum*. Fries' specimen of *Sphaeria mutabilis* and Quélet's specimen of *Lasiella mutabilis* are from UPS.

Collections were examined with a binocular microscope after mounting in 5% KOH. Spore measurements were made under a 100× oil immersion objective. Micrographs were taken with a Nikon (Eclipse 80i) microscope and a digital camera Nikon (DS-5M).

Taxonomy

Taxonomy of the *Thyridiaceae*

Yue & Eriksson (1987) established the family *Thyridiaceae* for *Sinosphaeria* and *Thyridium*. *Sinosphaeria bambusicola,* found on bamboo in China, is characterized by yellowish stromata with immersed ascomata that react with KOH to produce a reddish color and by the presence of irregularly brown dictyospores with thick darkened septa. *Thyridium* differs in producing KOH negative stromata in which the immersed ascomata have convergent ostioles and uniformly brown dictyospores.

Eriksson & Yue (1989) recognized *Thyridium* with *Bivonella* and *Sinosphaeria* as synonyms. They compared *T. chrysomallum* (≡ *Bivonella chrysomalla,* = *Sinosphaeria bambusicola*) with *T. flavum* (Petch 1917), which has a yellow subcortical, KOH negative stroma, ostioles not uniformly convergent, and dark brown dictyospores. This resulted in a broadly defined genus that accommodated varying stromatal structures and ascospore characters.

Samuels & Rogerson (1989) described *Sinosphaeria lasiacidis* on *Lasiacis ligulata* Hitchc. & Chase (*Poaceae*) from French Guiana. They stated that their species differed from *S. bambusicola* mainly by the smaller ascospores [(9–)9.8–11.6(–12.6) × (4.5–)5–5.8(–6.3) µm versus (12.5–)14–16.5(–17.5) × (6–)6.5–7.5 µm for *S. bambusicola* (= *T. chrysomalla*)].

Barr (1990) did not consider *Bivonella* to be a synonym of *Thyridium* because the type species of *Bivonella* (= *Sinosphaeria*) has periphysoids, which she could not find in *T. vestitum*. She accepted *Thyridiaceae* for these two genera and added *Valsaria* Ces. & De Not. and *Valsonectria* Speg., both with uniseptate ascospores. *Valsaria* currently falls within the *Diaporthales* and *Valsonectria* in the *Hypocreales* (Kirk et al. 2008).

Rossman et al. (1999) separated *Balzania*, *Mattirolia* and *Thyronectroidea* from the *Hypocreales* based on the paraphyses and included them in the *Thyridiaceae*. They also transferred *Calyptronectria ohiensis* to *Thyridium* as *T. ohiense*.

Genera included in *Thyridiaceae*

After studying numerous type specimens we accept the *Thyridiaceae* with two genera, *Mattirolia* (with five species) and *Thyridium* (with four species). A key to these genera and species is provided below.

Mattirolia Berl. & Bres., Annuario Soc. Alpin. Trident. 14: 351 (1889)

Type species: *Mattirolia roseovirens* Berl. & Bres.

= *Thyronectroidea* Seaver, Mycologia 1: 206 (1909)

Type species: *Thyronectroidea chrysogramma* (Ellis & Everh.) Seaver [≡ *Mattirolia chrysogramma*].

= *Balzania* Speg., Anales Mus. Nac. Buenos Aires 6: 286 (1898)

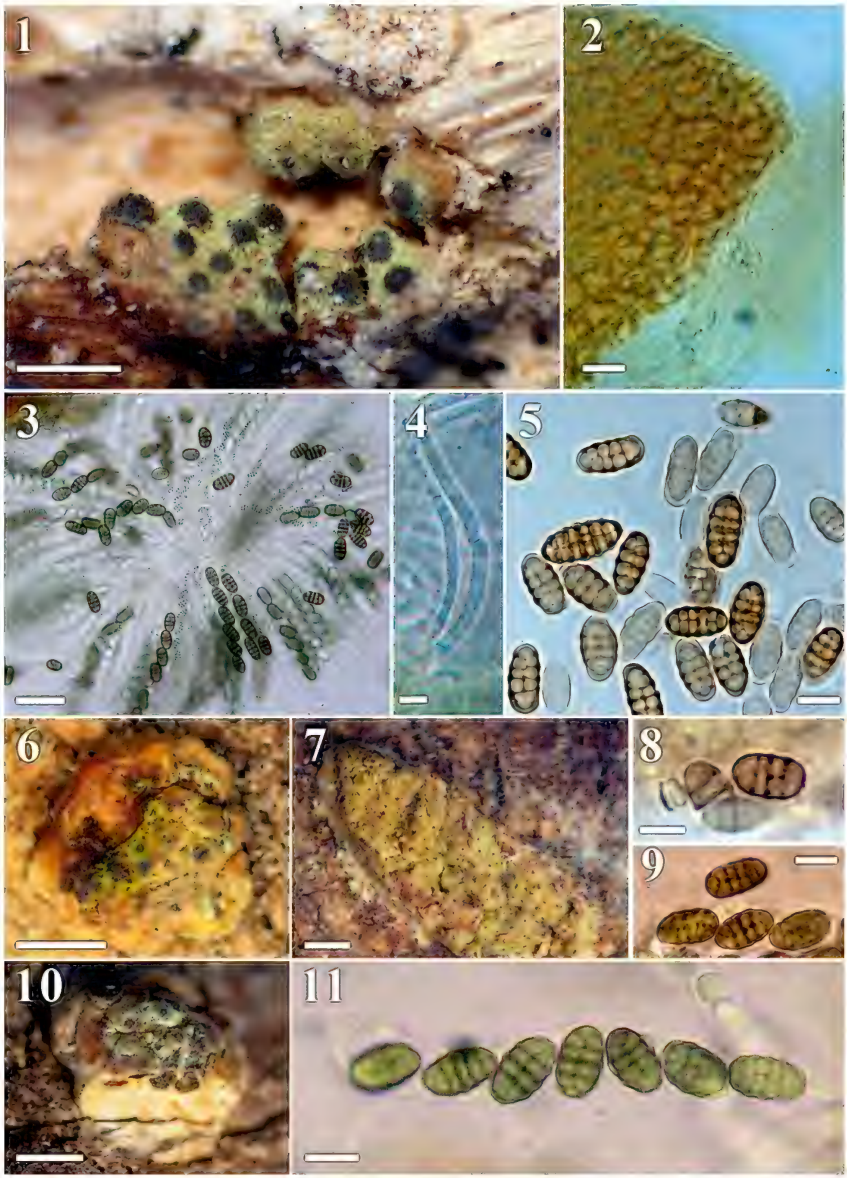
Type species: *Balzania platensis* Speg. [≡ *Mattirolia platensis*].

Stroma variable, usually present, erumpent, covered with loosely interwoven yellowish or brownish hyphae, KOH negative. Perithecia globose, semi-immersed or isolated in the stroma. Paraphyses abundant. Asci unitunicate, cylindrical or clavate, non amyloid. Ascospores smooth, muriform, hyaline to greenish yellow when mature.

Mattirolia roseovirens Berl. & Bres., Annuario Soc. Alpin. Trident. 14: 351 (1889) Figs. 1–11

Type: ITALY. Trento, Muralta, on branches of *Cytisus laburnum*, 14 April 1888, leg. G. Bresadola (type F115389 in S); FH 258826 (Patouillard sheet number 6749, probably an isotype).

Stroma subcortical, pulvinate, 0.5–6 mm diam. Perithecia aggregated, immersed in the stroma or more rarely isolated, globose, 400–450 µm diam., black, surrounded by a yellowish tomentum, 30–50 µm thick, formed by hyphae 4–5 µm diam. Peridium pseudoparenchymatous, orange, composed of isodiametric cells, 5–15 µm diam. Paraphyses 5 µm diam, regularly septate, sometimes with free ends. Asci unitunicate, cylindric, 100–120 × 12–15 µm, with 8 uniseriate ascospores. Ascospores greenish-brown, ellipsoid to oblong, 18–21 × 9–11 µm, with 3–5(–7) non-constricted, transverse septa and



Figs. 1–11. ***Mattirolia roseovirens*** (AH 39966): 1. Surface of stroma and perithecium. 2. Peridial cells and hyphae of the tomentum. 3. Asci and ascospores. 4. Paraphyses. 5. Ascospores. ***M. roseovirens*** (FH 258826): 6–7. Stromata. 8–9. Ascospores. ***M. roseovirens*** (S 115389 type): 10. Stroma. 11. Ascus with ascospores. Scale bars: 1 = 0.5 mm; 2, 4, 5, 8, 9, 11 = 10 µm; 3 = 50 µm; 6, 7, 10 = 1 mm.

1 longitudinal septum that includes the end cells, smooth and surrounded by a gelatinous sheath sometimes located only around ends.

Additional specimens examined, **ITALY. Veneto**, Patavii, 1889, leg. P.A. Saccardo, det. Berlese (BPI 553005 ex Herb. Saccardo [labelled as ex type, but specimen data do not correspond to type]); **Trentino**, Trento, in ramis corticalis Cytisi laburni, II-1890 (S ex Herb. Bresadola F115390, ex Herb. Mycolog. Lars Romell. F115391, F115392). **SPAIN. Guadalajara,** Tamajón, Enebrales´s hermitage, on branches of *Genista scorpius*, 18-V-1996, leg. H.O. Baral (AH 39966); 25-IV-2008, leg. J. Checa & M.N. Blanco (AH 39959, AH 39960, AH 39961, AH 39962, AH 39963); 22-V-2008 (AH 39233, AH 39964, AH 39965, AH 39967).

Remarks— This species is known from two related hosts in the *Fabaceae, Cytisus laburnum* and *Genista scorpius*.

Mattirolia chrysogramma (Ellis & Everh.) Sacc., Syll. Fung. 9: 993 (1891) Figs 12–16

≡ *Thyronectria chrysogramma* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 42: 245 (1890)

≡ *Thyronectroidea chrysogramma* (Ellis & Everh.) Seaver, Mycologia 1: 206 (1909)

≡ *Nectria chrysogramma* (Ellis & Everh.) Rossman, Mem. New York Bot. Gard. 49: 259 (1989)

Specimens examined, **USA, Kansas**, Manhattan, on bark of *Ulmus americana*, March 1889, Kellerman & Swingle 1421 (NY 00927545, holotype); **New York**, Potsdam, on elm limbs, Ellis 286 (NY 00927565, paratype).

Remarks— This is the type species of *Thyronectroidea*. The holotype specimen is depauperate, but the paratype includes numerous mature ascomata (Rossman et al. 1999). The appearance of this species is similar to *Mattirolia roseovirens,* and the ascospores have a greenish color. We recognize this species as *M. chrysogramma* and consider *Thyronectroidea* a synonym of *Mattirolia,* as suggested by Rossman et al. (1999).

Mattirolia mutabilis (Sacc.) Checa, M.N. Blanco & G. Moreno, **comb. nov.**

MycoBank MB 805470 Figs 17–25

≡ *Pleosphaeria mutabilis* Sacc., Syll. Fung. 2: 306 (1883)

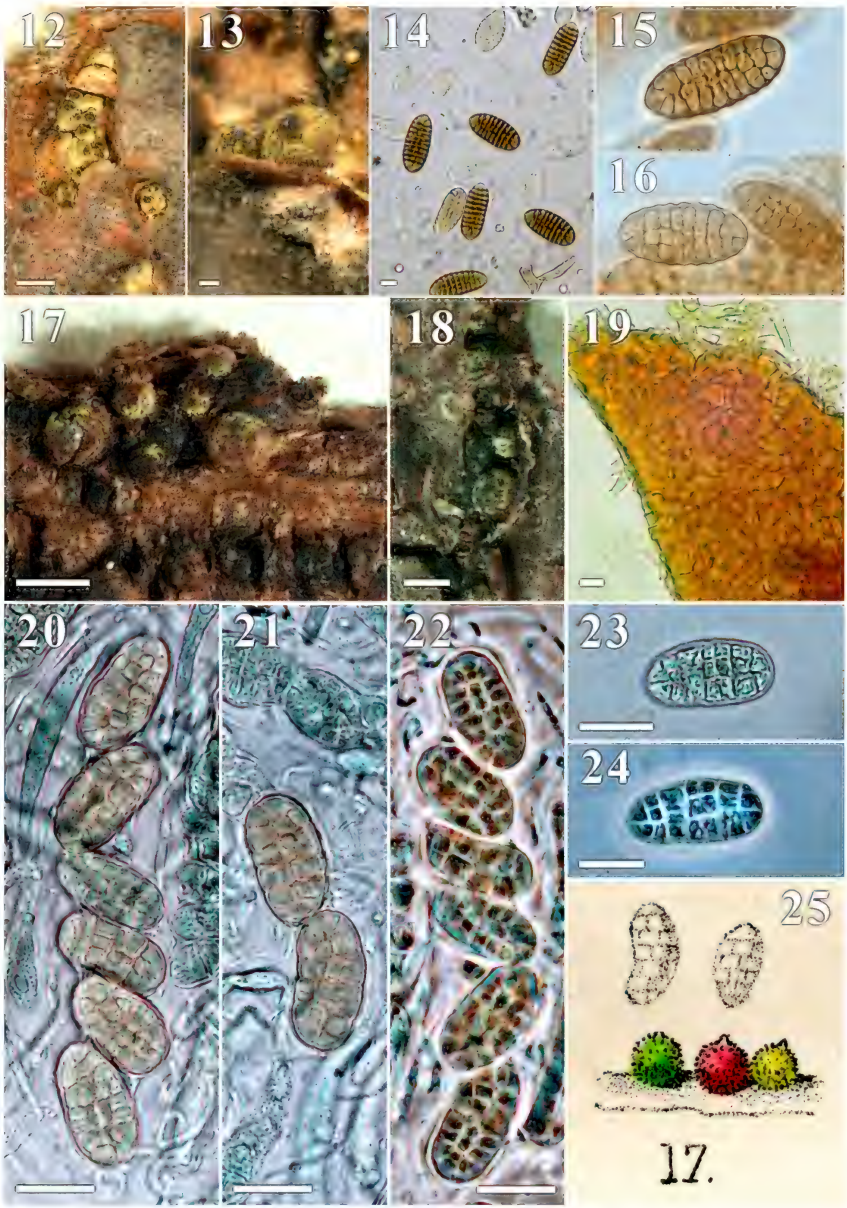
≡ *Strickeria mutabilis* (Sacc.) G. Winter, Rabenh. Krypt.-Fl., ed. 2, 1(2): 288 (1885)

Misapplied name: "*Lasiella mutabilis*" sensu Quélet, Mém. Soc. Émul. Montbéliard, 2e série, 5: 517 (1875)

Invalid name: "*Teichospora* cfr. *mutabilis*" Munk, Dansk Bot. Ark. 17(1): 394 (1957)

Type: FRANCE. Jura, La Bouloie, Saule marceau (*Salix*), leg. Lucien Quélet 406223 (**Lectotype designated here**, UPS F-126406, as *Sphaeria mutabilis*).

Stroma sometimes present. Perithecia subglobose to globose, 400–700 µm diam., superficial, scattered or aggregated, covered with a yellowish or yellow-brownish tomentum. Asci 80–90 × 10–15 µm, unitunicate, cylindrical-clavate, with 8 uniseriate ascospores or biseriate toward apex. Paraphyses 3–5 µm diam,



Figs. 12–25. ***Mattirolia chrysogramma*** (Ellis 286, NY 00927565 paratype): 12. Stromata. 13. Detail of stroma. 14. Ascospores. 15–16. Detail of ascospores. ***M. mutabilis*** (UPS 406223 (F-126406) lectotype): 17–18. Perithecia. 19. Peridial cells. 20–22. Asci with ascospores. 23–24. Detail of ascospores. ***M. mutabilis***: 25. Quélet's original drawing. Scale bars: 12, 17 = 1 mm; 13, 18 = 0.5 mm; 14–16, 19–24 = 10 µm.

abundant, hyaline, filiform, septate, cylindrical, with obtuse apex. Ascospores 19–22 × 8–12 µm, ellipsoid with rounded ends, muriform, 5–7 transverse septa and 1–2 longitudinal, pale septa, hyaline to pale yellow-greenish.

Remarks— *Mattirolia mutabilis* is characterized by unitunicate asci and hyaline to yellow-greenish muriform ascospores as well as isolated or grouped perithecia without a typical stroma; the perithecia are covered by a yellowish tomentum unlike in *Pseudotrichia mutabilis* (Table 1). Quélet (1875) recombined *Sphaeria mutabilis* as *Lasiella mutabilis*, but his interpretation and specimen do not match the original sense of Persoon's *S. mutabilis*, and this has caused later authors to misinterpret this name. Some authors have misinterpreted Quélet's publication of *Lasiella mutabilis* as creating a valid sp. nov. based on his description. However, this view is a nomenclatural error, because Quélet explicitly published *Lasiella mutabilis* as a valid and legitimate comb. nov. based on Persoon's *Sphaeria mutabilis;* he was therefore taxonomically misapplying this name when he attached it to his description of a different new species. Quélet's species acquired a valid name only when Saccardo (1883) renamed it as *Pleosphaeria mutabilis*, which is the correct basionym for all subsequent combinations. *Pleosphaeria mutabilis* is placed in the genus *Mattirolia* rather than *Lasiella,* now considered to be a synonym of *Lasiosphaeria* Ces. & De Not., because the ascospores of the latter are subcylindrical to sigmoid and have only transverse septa (Hilber & Hilber 1983; Miller & Huhndorf 2004; Munk 1957).

Mattirolia ohiensis (Ellis & Everh.) Checa, M.N. Blanco & G. Moreno, **comb. nov.**

MycoBank MB 805467 Figs 26–28

≡ *Teichospora ohiensis* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 46: 329 (1894)

≡ *Strickeria ohiensis* (Ellis & Everh.) Kuntze, Rev. Gen. Pl. 3(3): 534 (1898), as "*Strickera ohioensis*"

≡ *Calyptronectria ohiensis* (Ellis & Everh.) M.E. Barr, Mycotaxon 18: 155 (1983)

≡ *Thyridium ohiense* (Ellis & Everh.) Rossman & Samuels, Stud. Mycol. 42: 176 (1999)

Type, USA. Ohio, Preston, on hard dry wood, leg. A.P. Morgan 1012 (**Lectotype designated here**, NY Ellis collection, 00927519; **isolectotype**, NY 00927520).

Remarks— Barr (1983) placed this species in *Calyptronectria* and suggested that it could be related to *Thyronectria* but lacked a stroma. *Calyptronectria* is now placed in the *Melanommataceae* (Kirk et al. 2008). Based on our examination of the type specimen, this species belongs in *Mattirolia*.

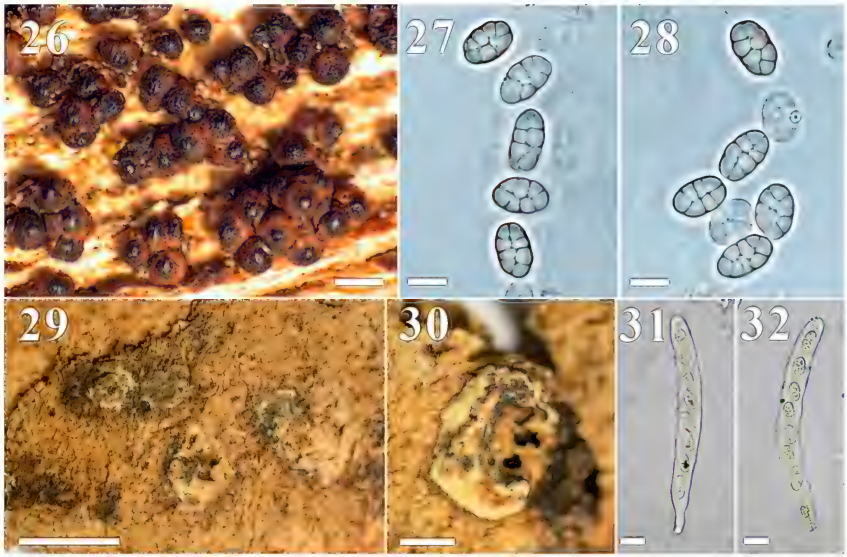
Mattirolia platensis (Speg.) Checa, M.N. Blanco & G. Moreno, **comb. nov.**

MycoBank MB 805468 Figs 29–32

≡ *Balzania platensis* Speg., Anales Mus. Nac. Buenos Aires 6: 286 (1898)

= *Mattirolia nivea* Speg., Anales Mus. Nac. Buenos Aires 6: 292 (1899)

≡ *Leucocrea nivea* (Speg.) Sacc. & P. Syd., Natürl. Pflanzenfam. 1(1**): 540 (1900)



Figs. 26–32. ***Mattirolia ohiensis*** (NY 927519, type): 26. Perithecia. 27–28. Detail of ascospores. ***M. platensis*** (LPS 1645, holotype): 29. Stromata. 30. Detail of stroma. 30. Ascospores. 31–32. Asci and ascospores. Scale bars: 26 = 0.5 mm; 27, 28 = 10 µm; 29 = 5 mm; 30 = 1 mm: 31, 32 = 20 µm.

Specimen examined, **ARGENTINA**. La Plata, on bark of dead trunks of *Ailanthus glandulosa*, Aug 1891, C. Spegazzini (LPS 1645, holotype).

Remarks—Although this specimen is in poor condition, we were able to observe it macro- and microscopically. The asci are unitunicate and the ascospores are muriform, hyaline to yellowish; thus we include it in *Mattirolia*.

The holotype specimen of *Mattirolia nivea* (LPS-1704) was examined and considered a synonym of *B. platensis* by Rossman et al. (1999).

Thyridium Nitschke, Pyrenomycetes Germanici 1: 110 (1867)

Type species: *Thyridium vestitum* (Fr.) Fuckel

= *Bivonella* (Sacc.) Sacc., Syll. Fung. 9: 989 (1891)

Type species: *Bivonella lycopersici* (Pass.) Sacc.

= *Sinosphaeria* J.Z. Yue & O.E. Erikss., Syst. Ascom. 6: 231 (1987)

Type species: *Sinosphaeria bambusicola* J.Z. Yue & O.E. Erikss.

Stromata present, immersed or erumpent, formed by interwoven yellowish, brownish or black hyphae and cells of the substrate, KOH positive (fuchsia) or KOH negative. Perithecia globose with short or long necks with convergent or separate ostioles, immersed. Asci cylindrical, inamyloid. Ascospores muriform, very pale brown to dark brown, or with thickened and pigmented septa.

Remarks— Eriksson & Yue (1989) emended *Thyridium* to incorporate *Bivonella* and *Sinosphaeria* as synonyms.

Thyridium vestitum (Fr.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 195 (1870) Figs 33–35

= *Fenestella ulmicola* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 45: 143 (1893)

Specimens examined, **AUSTRIA, Bohemia**, on *Sambucus racemosa*, 1873 (de Thümen 859, NY). **CANADA, Ontario**, London, on dead limbs of *Ulmus americana*, VIII-1892 (Holotype of *Fenestella ulmicola*-NY 00928688); on *Ribes lacustre*, 30-I-1894 (NY). **GERMANY**, on *Ribes rubrum*, VIII-1882, (NY); **Saxony**, Königstein, on *Sambucus racemosa*, XI-1882, leg. W. Krieger (de Thümen, 2254, NY); **Berlin**, on *Frangula alnus*, V-1887, leg. Sydow 2249, Mycotheca Marchica n. 1362 (NY); **Rhineland-Palatinate**, Oestrich (Nassau), on *Betula alba* and *Robinia pseudoacacia*, 1894, leg Fuckel (Herbier Barbey-Boissier, NY). **HUNGARY**, on *Colutea arborescens*, VI-1875, leg. Lojka (NY). **ITALIA, Veneto**, Treviso, Vitorio, on *Ribes grossularia*, IX-1897, (D. Saccardo 107, NY). **POLAND, Mazowieckie**, Warszawa-Goclawek, on *Elaeagnus angustifolia*, 25-IX-1960, leg. J. Kochman (J. Kochman Mycotheca Polonica in NY). **U.S.A. Iowa**, on *Carya* sp. (NY); **Massachusetts**, Baptist Hill, Conway, on old branch of *Berberis* sp., 23-XII-1979, coll. and det. M.E. Barr, 6652 (Herbarium of the University of Massachusetts in NY); on young dead branches of *Forsythia* sp., 15-IV-1961, coll. & det. M.E. Barr, 2872 (Herbarium of the University of Massachusetts in NY); Sunderland, on branches of *Betula* sp., 25-VIII-1963, coll. and det. M.E. Barr 4126 (NY). **Missouri**, on dead sprouts of *Platanus occidentalis*, 27-X-1897, leg. C.N. Demetrio (NY).

Remarks— Macroscopically this species is variable and occurs frequently on diverse substrates. A complete description with illustrations of macro- and microscopic characteristics appears in Barr (1983).

Thyridium chrysomallum (Berk. & Broome) O.E. Erikss. & J.Z. Yue, Syst. Ascom. 8: 12 (1989) Figs 36–37

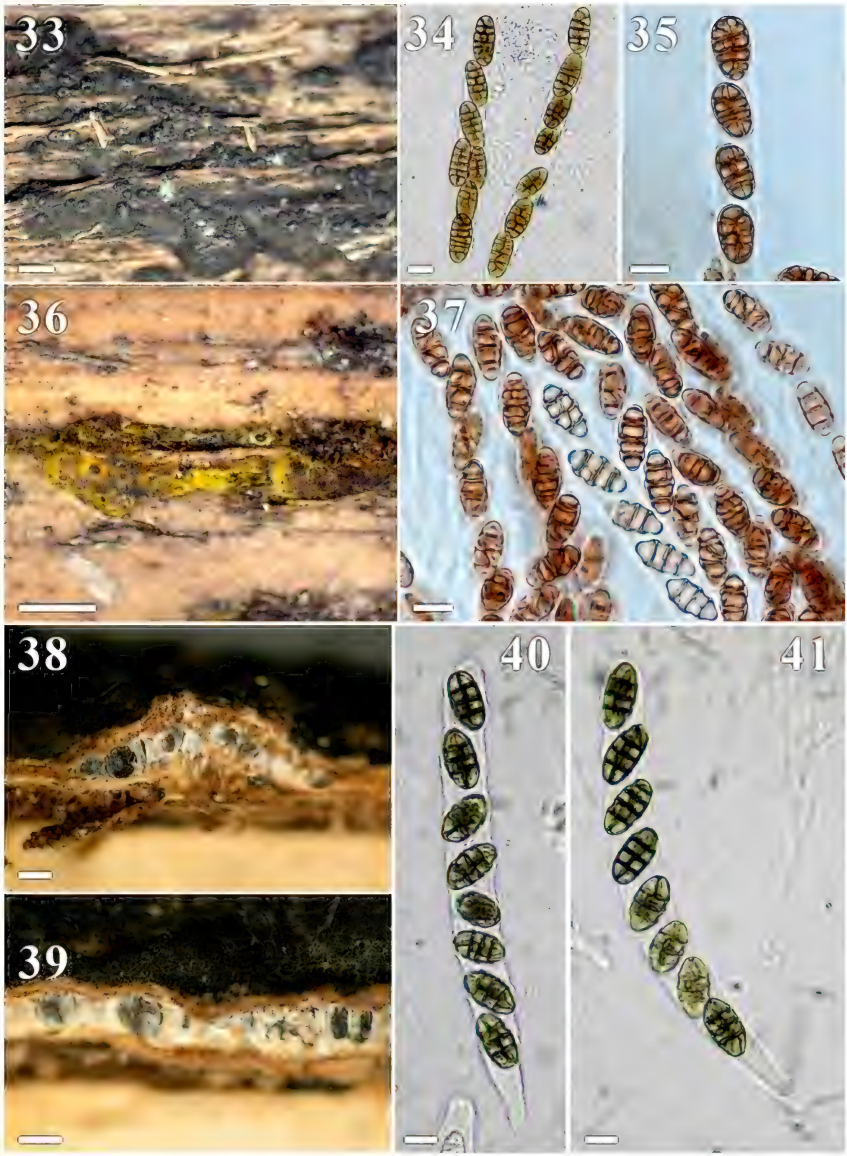
≡ *Melanospora chrysomalla* Berk. & Broome, J. Linn. Soc. Bot. 14: 130 (1873)

≡ *Bivonella chrysomalla* (Berk. & Broome) Sacc., Syll. Fung. 9: 990 (1891), as "*chrysomella*"

= *Sinosphaeria bambusicola* J.Z. Yue & O.E. Erikss. *Syst. Ascom.* 6: 231 (1987)

Specimens examined, **NEW ZEALAND, Bay of Plenty**, Te Puke, D.S.I.R. Research Orchard, on canes of *Actinidia deliciosa,* 15-VII-1981, coll. G.J. Samuels (81-209) & S.R. Pennycook, det. M.E. Barr [as *Thyridium vestitum* on *Actinidia chinensis*] (Herbarium of the University of Massachusetts in NY). **SRI LANKA**, Peradeniya, on palm stems, November 1868, coll. G.H.K. Thwaites 1071 (K(M) 36218, ex herb. M.J. Berkeley, holotype of *Melanospora chrysomalla*). **VENEZUELA, Aragua**, on Colonia Tovar-La Victoria road, ca 7 km from Colonia Tovar, on unidentified bamboo, 14-VII-1971, leg. K.P. Dumont, J.H. Haines & G.J. Samuels (NY, Dumont-VE 2192).

Remarks— Macroscopically this species is similar to *Mattirolia roseovirens* and also has ascospores with very thick septa but their color is pale brown and not greenish. The stroma reacts positively with KOH taking on a fuchsia color.



Figs. 33–41. ***Thyridium vestitum***: 33. Stroma (Thümen, 2249 in NY). 34–35. Asci and ascospores (M.E. Barr 2872 in NY). ***T. chrysomallum*** (NY, Dumont-VE 2192): 36. Stroma. 37. Ascospores. ***T. flavum*** (isotype K(M) 160957): 38–39. Stromata. 40–41. Asci and ascospores. Scale bars: 33 = 1 mm; 34, 35, 37, 40, 41 = 10 µm; 36, 38, 39 = 0.5 mm.

The sequences of *Thyridium chrysomallum* (as *Sinosphaeria bambusicola*) in Genbank have been shown to be erroneous and have been removed (Miller, pers. comm.), thus the taxonomic position of this species is not known.

Thyridium flavum Petch, Ann. Roy. Bot. Gard. Peradeniya 6(3): 226 (1917)
FIGS. 38–41

SPECIMEN EXAMINED, **SRI LANKA**, Peradeniya, on twigs of *Hevea brasiliensis*, February 1917, coll. Petch 4968 (isotype, K(M) 160957).

REMARKS— The KOH– perithecia in the isotype specimen are blackish (lacking a yellowish color), short-necked, and grouped in rows under the bark of a *Hevea brasiliensis* branch. Under the microscope we observed cylindrical, unitunicate asci and dark brown ellipsoid ascospores, 16–20 × 8–10 µm, with 3(–5) transverse septa and 1 longitudinal septum, which correspond to the description by Petch (1917). We also observed many ascospores on the bark of the substrate giving the false appearance of conidia or formation of proliferate asci, a feature not noted by the author nor found in other species of *Thyridiaceae*.

Thyridium lasiacidis (Samuels & Rogerson) Checa, M.N. Blanco & G. Moreno, **comb. nov.** FIGS 42–45

MYCOBANK MB 805474

≡ *Sinosphaeria lasiacidis* Samuels & Rogerson, Stud. Mycol. 31: 147 (1989)

SPECIMENS EXAMINED, **FRENCH GUIANA**, Saül, 200 m, on dead culms of *Lasiacis ligulata*, 3-II-1986, leg. G.J. Samuels 3784A (Holotype, NY); II-1986, leg. G.J. Samuels 3773 (UME 33336).

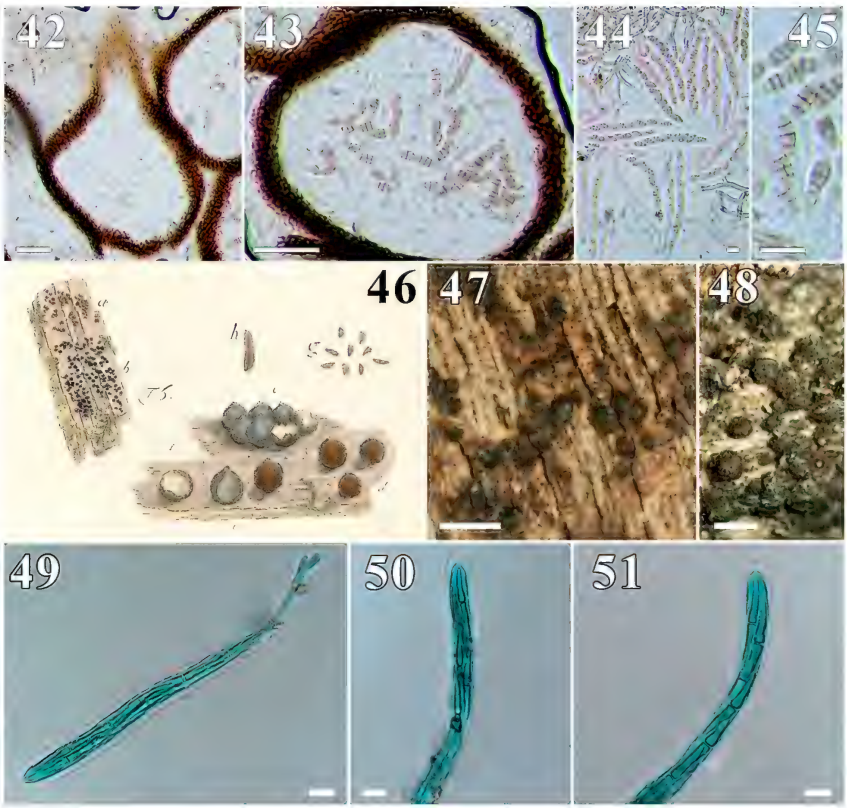
STROMA present, KOH positive. ASCI clavate to broadly cylindrical. ASCOSPORES very pale brown with 3 transverse septa and 1 longitudinal septum in median cells that are not conspicuously darkened.

REMARKS— Because *Sinosphaeria* is considered a synonym of *Thyridium,* this species is transferred to *Thyridium*. It is similar to *T. chrysomallum* but has smaller spores (10–12 × 5–6 µm).

Key to species of *Thyridiaceae*

1. Stroma usually present, erumpent, with a loosely interwoven yellowish or brownish tomentum, KOH negative; perithecia globose without convergent ostioles; ascospores muriform, hyaline to greenish yellow when mature (***Mattirolia***) **2**

1. Stroma always present, immersed or erumpent, with interwoven yellowish, brownish or black tomentum and cells of the substrate, KOH negative or positive; perithecia globose with short or long necks, sometimes convergent ostioles; ascospores muriform, very pale brown to dark brown, or with wall thickened and pigmented only at the septa (***Thyridium***) **6**



Figs. 42–51 ***Thyridium lasiacidis*** (NSF BSR 8500236 holotype in NY): 42. Detail of the stroma. 43. Perithecium. 44. Asci and ascospores. 45. Detail of ascospores. ***Pseudotrichia mutabilis***: 46. Persoon's original drawing of *Sphaeria mutabilis* (tab. 7, fig. 6a, 6d, 6e). ***P. mutabilis*** (B 700014106): 47–48. Pseudothecia. 49. Ascus. 50–51. Detail of asci and ascospores. Scale bars: 42, 43 = 50 µm; 44, 45, 49–51 = 10 µm; 47 = 1 mm; 48 = 0.5 mm.

2. Perithecia solitary, surrounded with brownish tomentum; ascospores 3–4 transverse septa and 1 irregularly longitudinal septum ..... ***M. ohiensis***
2. Perithecia semi-immersed in a stroma or solitary, with yellowish tomentum 3
3. Ascospores with 3 transverse septa ..... ***M. platensis***
3. Ascospores with >3 transverse septa 4
4. Ascospores with 3–5(–7) transverse septa ..... ***M. roseovirens***
4. Ascospores with more than 5 transverse septa 5
5. Ascospores with 5–7 transverse septa ..... ***M. mutabilis***
5. Ascospores with 7–9(–11) transverse septa ..... ***M. chrysogramma***

6. Stroma variable in size and sometimes in valsoid configuration, KOH negative; ascospores uniformly brown 7
6. Stroma not in valsoid configuration KOH positive; ascospores with 3 transverse septa and 1 longitudinal septum, thickened, not uniformly brown 8
7. Ascospores with 3–5(–8) transverse septa ..... ***T. vestitum***
7. Ascospores with fewer [3(–5)] transverse septa ..... ***T. flavum***
8. Ascospores 10–12 × 5–6 µm ..... ***T. lasiacidis***
8. Ascospores (12.5–)14–16.5(–17.5) × (6–)6.5–7.5 µm ..... ***T. chrysomallum***

Nomenclatural problem with *Sphaeria mutabilis*

In his description and illustration of *Sphaeria mutabilis,* Persoon (1798, 1801) provided few microscopic characters. His drawings, which are too small to illustrate detailed morphology, do not include ascospores. In addition, he did not provide ascospore measurements or identify the substrate. Fries (1823), who studied Persoon's specimen, observed that the specimens grew "ad ligna durisima quercina, ad terram prostata". Later, Barr (1990) studied Fries' material and gave a macro- and microscopic description of this species (see Table 1). Our observations of Fries' material agree with those of Barr (1990). This species is now regarded as *Pseudotrichia mutabilis* in the *Pleosporales.*

Quélet (1875) transferred *Sphaeria mutabilis* to *Lasiella,* but misapplied this name to a specimen growing on woody debris of willows and oak (now recognized as *Mattirolia mutabilis*). The distinction between Persoon's and Quélet's taxa was quickly recognised by Saccardo (1883: *Lasiosphaeria mutabilis* [p. 196] versus *Pleosphaeria mutabilis* [p. 306]) and by Winter (1884–87: *Herpotrichia mutabilis* [p. 209] versus *Strickeria mutabilis* [p. 288]).

Pseudotrichia mutabilis (Pers.) Wehm., Fungi of Maritime Provinces: 35 (1950)
Figs 46–51

≡ *Sphaeria mutabilis* Pers., Icon. descr. Fung. 1: 24 (1798)
≡ *Lasiosphaeria mutabilis* (Pers.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 25–26: 302 (1871)
≡ *Lasiella mutabilis* (Pers.) Quél. [non sensu Quélet], Mém. Soc. Émul. Montbéliard, 2e série, 5: 517 (1875)
≡ *Herpotrichia mutabilis* (Pers.) G. Winter, Rabenh. Krypt.-Fl., Ed. 2, 1(2): 209 (1885)
≡ *Enchnosphaeria mutabilis* (Pers.) Höhn., Sitzungsber. K. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 126: 346 (1917)
≡ *Khekia mutabilis* (Pers.) Petr., Ann. Mycol. 38: 203 (1940)

Specimens examined, **GERMANY**: **Lothringen**, [now = **FRANCE**: **Lorraine**, Moselle], Forbach, Schönecker wald, 26-IX-1913, *leg.* Dr. A. Ludwig, (B 700014106 as *Herpotrichia mutabilis*). **SWEDEN**: **Småland**, Femsjö, Herb. E. Fries (UPS F-07198, 133504); **Gästrikland**, Gävle, Lövudden, on the bank of the R. Gavleån, *Alnus* (?), ramus dejectus, 7-I-1973, leg. J. Ax. Nannfeldt n° 22906 (UPS F-126407, 406224 as

Herpotrichia mutabilis); in ramis dejectis, 17-VIII-1950, leg. J. Ax. Nannfeldt nº 11084, (UPS F-126408, 406225 as *Lasiosphaeria*, rev. G. Carroll 1963 as "*Lophiostoma mutabilis*". **USA: Alabama**, Cort. Cerasi Caroliniani, *leg.* Beaumont ex. Herb. M.A. Curtis (UPS F-04319, 57500, Herb. E. Fries).

Remarks— Although Persoon (1798) did not describe the hamathecium, asci, and ascospores verbally in his protologue, he did include them in his drawing. We provide a description of those features in Table 1.

Table 1. A morphological comparison of *Pseudotrichia mutabilis* and *Mattirolia mutabilis*.

	*P. mutabilis** UPS (F-01798) 133504	*M. mutabilis*** UPS (F-126406) 406223
Ascomata	Scattered or aggregated; (Stroma not observed); Tomentum brown-yellowish; Ostiole papillate	Scattered or aggregated; (Stroma not observed); Tomentum yellow to yellow-greenish; Ostiole subpapillate and blackish
Asci	**Bitunicate,** clavate, (90–)120–155 × 12–20 µm	**Unitunicate,** Cylindrical-clavate, 80–90 × 10–15 µm.
Hamathecium	**Trabecular pseudoparaphyses**	**Paraphyses**
Ascospores	26–39 × (6–)7–9 µm, ellipsoid-fusiform **1–3 tranverse septa** **0 longitudinal septa** Hyaline to light brown.	19–22 × 8–12 µm, ellipsoid **5–7 transverse septa** **1–2 longitudinal septa** Hyaline to lightly yellow-greenish

* Description from Barr (1990) based on material collected by Fries from Sweden.
** Data from this paper.

Barr (1984) provided a comprehensive description and illustration of *Pseudotrichia mutabilis,* although she did not study the type specimen.

Conclusions

After examining the type species of *Balzania*, *Mattirolia*, *Thyridium*, and *Thyronectroidea*, we accept only *Mattirolia* (with *Balzania* and *Thyronectroidea* in synonymy) and *Thyridium* within the *Thyridiaceae*. We can define *Mattirolia* by a stroma that is generally present, KOH negative, cylindrical to clavate asci, and muriform ascospores that become yellow-green when mature. *Thyridium* is characterized by the presence of stromata, KOH variable, cylindrical or clavate asci, and very pale brown to dark brown muriform ascospores that lack green tones.

Based on our examination of the type specimens, *Thyridium ohiense* does not belong in *Thyridium* due to the absence of stroma and the presence of hyaline or yellowish spores. Its macro- and microscopic characteristics place it instead in *Mattirolia.*

Balzania, a monotypic genus described more than 100 years ago by Spegazzini (1898) for *Balzania platensis*, which has been collected only once, must be included within *Mattirolia*, as Rossman et al. (1999) suggested. *Thyronectroidea* is also a monotypic genus, which we consider synonymous with *Mattirolia*. The type species, *T. chrysogramma,* appears macroscopically similar to *M. roseovirens,* and the ascospores are hyaline to faintly greenish when mature. As these two species are congeneric, we use the name *M. chrysogramma.*

Because of Quélet's (1875) misapplication of the name, *Lasiella mutabilis* has been misinterpreted by different authors. The earliest valid name for Quélet's species was *Pleosphaeria mutabilis*, here recombined as *Mattirolia mutabilis* in the *Thyridiaceae; Pseudotrichia mutabilis,* based on Persoon's *Sphaeria mutabilis,* belongs in the order *Pleosporales.*

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Phyllosticta ephedricola sp. nov. on *Ephedra intermedia*

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Abstract — The new anamorphic species *Phyllosticta ephedricola* on *Ephedra intermedia* in China is described, illustrated, and differentiated from *P. ephedrae*.

Key words — coelomycete, pathogen, taxonomy

Introduction

A species of *Phyllosticta* on necrotic stem spots of *Ephedra intermedia* was collected in 2011 from Hami, Xinjiang Province, China. Severe infections caused by this pathogenic fungus usually result in death of ground parts of the plants. Two characteristics, the shape of the conidiogenous cells and the cylindrical to clavate apically appendaged conidia, indicate that the fungus is a species of *Phyllosticta*. As no authentic *Phyllosticta* species has previously been described from *Ephedra* (Aa & Vanev 2002), we propose it as a new species.

Materials & methods

The fungal specimens were cut by hand using a razor blade to make thin sections of conidiomata for microscope observation. They were examined under a light microscope after they were mounted in water and lactophenol cotton blue on slides. Measurements for the new species were determined using the means of 50 spores and at least 20 pycnidia. The examined specimens were deposited in Lanzhou University Herbarium (LZU), Lanzhou, Gansu Province, China.

Taxonomy

Phyllosticta ephedricola L. Jin & Yan Wang, **sp. nov.** Plate 1

MycoBank MB 803189

Differs from all other *Phyllosticta* species by its ephedraceous host.

Type: China, Xinjiang Province, Hami city, on stems of *Ephedra intermedia* Schrenk & C.A. Mey. (*Ephedraceae*), 22 July 2011, L. Jin (**Holotype**, LZU 11001214).

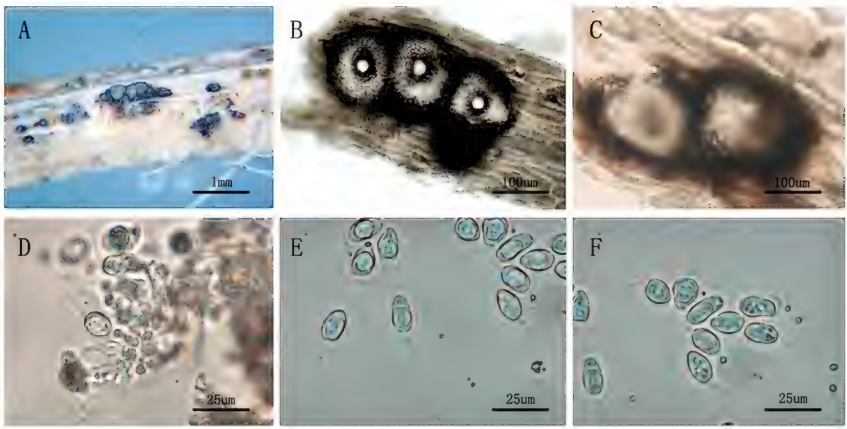

Plate 1. *Phyllosticta ephedricola* (holotype). A: Dark-brown stromata on stem of *Ephedra intermedia*. B: Ostioles. C: Section through a stroma showing an immersed eustromatic conidioma with inner surface covered by a layer of conidiogenous cells bearing conidia. D: Conidiogenous cell bearing an appendage-bearing conidium. E–F: Typical appendage-bearing guttulate conidia. Scale bars: A = 1 mm; B–C = 100 µm; D–F = 25 µm.

Etymology: Epithet refers to the host genus.

Occurring on stems. Mycelium immersed in the host tissue, septate, branched, hyaline to pale brown. Pycnidia immersed, or semi-immersed, separate or aggregated, globose to subglobose, 227.6 × 231.0 µm diam., brown to dark brown. Ostioles single, central, circular, 31.3 × 35.3 µm diam., with darker and thicker walls around the pore. Wall 20–32 µm diam., composed of 3–4 layers of small, rounded or irregular, pale brown to dark brown thick-walled cells and an inner textura prismatica with thin-walled paler cells. Conidiogenous cells hyaline, cylindrical, discrete, determinate, smooth, forming one apical conidium, 8.8 × 2.5 µm diam., originated from the internal layer of conidiomatal wall, sometimes hardly differentiated from the flat inner wall-cells. Conidia one-celled, ellipsoidal or obovoidal, seldom cylindrical, sometimes pyriform with a truncate base, broadly rounded apically and inconspicuously indented, 9.4–15.8 × 5.4–12.8 µm, surrounded by a slime layer, containing one large, bright guttule, 9.1–14.5 × 7.2–11.5 µm, almost fill the whole conidium. Apical appendage 12.5–34.4 × 1.9–3.3 µm, hyaline, single, subulate or filiform gelatinous, filiform, cellular, with subacute apex. The spermatial synanamorph and the *Guignardia* teleomorph have not been observed.

Discussion

The genus *Phyllosticta* Pers. (with type *P. convallariae* Pers.; Donk 1964) was introduced by Persoon in 1818. The modern generic circumscription is restricted to species with large unicellular guttulate conidia with a slime

layer and apical extracellular appendage and teleomorphs (where known) in *Guignardia* Viala & Ravaz (Aa 1973; Nag Raj 1993; Aa & Vanev 2002). Aa & Vanev (2002) accepted 141 species in *Phyllosticta* sensu stricto, while Kirk et al. (2008) estimated that there were only 92 species; recently another nine species have been described (Motohashi et al. 2008; Wikee et al. 2011).

Only one other *Phyllosticta* species has been described from *Ephedra*: *P. ephedrae* Trotter on *E. altissima* Desf. (Trotter 1916: 21). However, Aa & Vanev (2002: 200), who did not accept *P. ephedrae* as an authentic *Phyllosticta,* suggested that its small eguttulate conidia without appendages indicated that it was an *Asteromella* species (possibly the spermatial state of one of the five *Mycosphaerella* species described from *Ephedra*). Since *Ephedra* is a botanically isolated gymnospermous genus in a monogeneric family and order, we consider that the host specificity of *P. ephedricola* differentiates it from all other accepted *Phyllosticta* species.

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Scutellospora alterata, a new gigasporalean species from the semi-arid Caatinga biome in Northeastern Brazil

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Abstract — A new species of *Gigasporales* (*Glomeromycota*) was isolated from soils of the semi-arid Caatinga biome in NE Brazil. It produced spores in the rhizospheres of *Sorghum sudanense*, *Zea mays*, *Panicum maximum*, and *Helianthus annuus* in pot cultures. Spores are triple-walled, roughened, yellow-white to light yellow, and 150–255 µm in diameter. The single germination shield is hyaline, oval to ovoid, and bi-lobed and has the two germ tube initiations (GTIs) typical for *Scutellospora* species. The shields can change shape under light pressure on the cover slide, causing several 'false' lobes to appear that do not bear GTIs but inflate from the shield periphery. Molecular analyses of the partial LSU rDNA gene place the fungus in a clade next to *Scutellospora calospora*, *S. dipurpurescens,* and *S. spinosissima*. Further investigations on germ shield morphology showed that several false lobes also form in other *Scutellospora* species in lactic acid based mountants under pressure on the cover slide. In *Racocetraceae* more than two 'true' lobes form during spore formation, each potentially bearing one GTI.

Key words — *Glomeromycetes*, *Scutellosporaceae*, *Orbispora*, molecular phylogeny, arbuscular mycorrhiza

Introduction

During the last decades a high species richness of arbuscular mycorrhizal fungi (AMF) has been detected in the *Gigasporales* (e.g., Nicolson & Gerdemann 1968, Gerdemann & Trappe 1974, Ferrer & Herrera 1981, Morton

1995, Souza et al. 2005, Silva et al. 2006, Tchabi et al. 2009a). Many species have been described within this fungal group (e.g., Koske & Halvorson 1990, Błaszkowski 1991, Lin & Yen 2011), especially from tropical South America (e.g., Spain et al. 1989, Walker et al. 1998, Cuenca & Herrera-Peraza 2008, Silva et al. 2008, Goto et al. 2012, Mello et al. 2012). The morphological diversity in the *Gigasporales* concerns above all spore sizes and color (Silva et al. 2008, Goto et al. 2011), outer spore wall ornamentation (Goto et al. 2009, 2010, Tchabi et al. 2009b), number of walls (Oehl et al. 2008, Oehl et al. 2011a), germination shield complexity and color, and specific germination characteristics (Oehl et al. 2010, Oehl et al. 2011a,c,d, Goto et al. 2012). Within the *Gigasporales*, major fungal groups have been identified as a result of detailed morphological and molecular phylogenetic analyses. These analyses so far support five families and ten genera (Silva et al. 2013).

A new species of the *Gigasporales* producing spores with an evanescent papillate surface roughening has been isolated from tropical soils of the semi-arid 'Caatinga' biome in NE Brazil. The fungus forms triple-walled spores with hyaline to subhyaline bi-lobed germination shields, clearly suggesting placement in *Scutellospora* (*Scutellosporaceae*) according to Oehl et al. (2011c). However, we observed an atypically highly variable germination shield morphology, especially when we applied pressure on the cover slides in lactic acid based mountants. The aim of this study was to a) describe this unique species, b) study thoroughly its variable shield morphology, and c) analyze its ribosomal gene. Shields in other *Scutellospora* species were reviewed, and their morphological variation is also discussed.

Materials & methods

Study site, soil sampling and chemical soil analyses

The study site was located at 833 m asl at the Research Station of the Instituto de Pesquisas Agronômicas (IPA) in Araripina (7°29′S 40°36′W), Pernambuco State, NE Brazil, in natural vegetation in the semi-arid 'Caatinga' biome. The mean annual rainfall is 742 mm, temperature is 23°C, and humidity is 52%. According to Albuquerque (2008), the native Caatinga vegetation at the site is hyperxerophilic with *Bauhinia cheilantha* (Bong.) Steud., *Croton sonderianus* Müll. Arg., *Spondias tuberosa* Arruda, and *Mimosa malacocentra* (Mart.) Benth. dominating.

Field soil samples (0–20 cm depth) were taken in October 2010 and analyzed for selected chemical soil characteristics as described in Goto et al. (2009). Mean soil pH (H_2O) was 4.7, organic carbon 17.1 $g.kg^{-1}$, and available P was 63 $mg.kg^{-1}$.

AMF bait cultures

Soil from Araripina was placed in four 1 L pots under greenhouse conditions at the Department of Mycology, Universidade Federal de Pernambuco (Recife) as described in Mello et al. (2012) with the objective to cultivate and reproduce the native AMF communities. Corn (*Zea mays* L.), *Sorghum sudanense* (Piper) Stapf, *Panicum maximum*

Jacq., and *Helianthus annuus* L. were planted as host plant mixtures in the same pots during three months. For propagation, field samples of the five field soil replicates were combined into one homogenous AMF inoculum. The new fungus sporulated abundantly in two pots co-occurring with AMF species like *Acaulospora sieverdingii*, *A. spinosa*, *Ambispora appendicula*, *Dentiscutata cerradensis*, and *Gigaspora gigantea*. Despite several attempts, single species cultures of the new fungal species could not be established. Voucher specimens were deposited in the herbaria of Eidgenössische Technische Hochschule, Zürich, Switzerland (ZT), and Universidade Federal de Pernambuco, Recife, Brazil (URM).

Morphological analyses

About 200 spores were extracted from the field soils and bait cultures by wet sieving and sucrose centrifugation (Sieverding 1991). The spores were mounted in polyvinyl-alcohol–lactic acid–glycerin (PVLG), in PVLG + Melzer's reagent, and in water, and microscopically examined. The terminology of Oehl et al. (2008, 2011a) for spore morphology and germination of gigasporalean species is followed.

Molecular analyses

PCR reactions were performed on DNA extracted from five surface-sterilized spores (Mosse 1962) isolated from the bait cultures and crushed with a sterile disposable micropestle in an Eppendorf tube with 23 µL of milli-Q water.

A two-step PCR was conducted to amplify the ribosomal fragment consisting of partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr consecutively according to Krüger et al. (2009). PCR reactions were carried out in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, California) with a pureTaq Ready-To-Go PCR Beads (Amersham Biosciences Europe GmbH, Germany) following manufacturer's instructions with 0.4 µM concentration of each primer. PCR products were analyzed by electrophoresis in 1.2% agarose gels, stained with Gel Red™ (Biotium Inc., Hayward, CA, U.S.A.) and viewed by UV illumination. The expected amplicons were purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ), cloned into the pCR2.1 vector (Invitrogen, Carlsbad, California), and transformed into one shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen, Carlsbad, California). After plasmid isolation from transformed cells, cloned DNA fragments were sequenced with vector primers (White et al. 1990) in both directions by Taq polymerase cycle sequencing on an automated DNA sequencer (Perkin-Elmer ABI Prism 373).

Querying the National Center for Biotechnology Information databases with the BLASTn program, we verified that the sequences obtained from the new fungus were affiliated with the *Gigasporales* (*Glomeromycota*). The AMF sequences (partial LSU rRNA) obtained were aligned with other glomeromycotan sequences from GenBank using the program ClustalX (Larkin et al. 2007) and edited with the BioEdit program (Hall 1999) to obtain a final alignment. The sequences were deposited at GenBank under the accession numbers HF935017, HF935020–HF935022, and HF935024.

Maximum parsimony (MP) and neighbor joining (NJ) analyses with 1000 bootstrap replications were performed using the Phylogenetic Analysis Using Parsimony (PAUP) program version 4 (Swofford 2003). Bayesian (two runs over 1×10^6 generations with a

burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The model of nucleotide substitution (GTR + G) was estimated using Topali 2.5 (Milne et al. 2004). Two sequences from *Pacispora scintillans* (FM876831, FM876832) were used as outgroup.

Results

Taxonomic analyses

Scutellospora alterata Oehl, J.S. Pontes, Palenz., Sánchez-Castro & G.A. Silva, **sp. nov.** FIGS 1–18

MYCOBANK MB 803603

Differs from all other *Scutellospora* spp. by the evanescent papillate surface roughening on the spores.

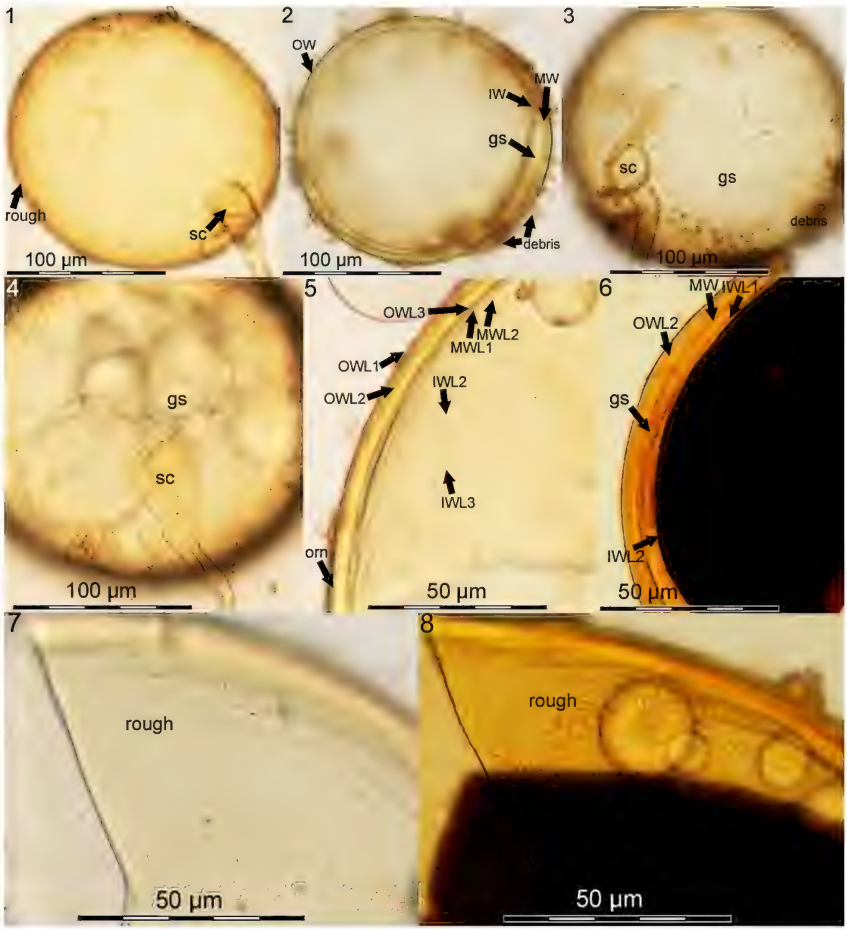
TYPE: Brazil, Pernambuco, Araripina, isolated from pot cultures on plants inoculated with soils from natural Caatinga, 94-9401 (**holotype**, ZT Myc 35647); 94-9402–9410 (**isotypes**, ZT Myc 35648); 94-9411–9413 (**isotypes**, URM 83532–URM 83534).

ETYMOLOGY: *alterata* (altered), referring to the variable germ shield shape, depending on the pressure applied on cover slides.

GLOMEROSPORES are singly formed in soils terminally on single, bulbous suspensor cells (= 'sporogenous' cells). Glomerospores are yellowish white to bright yellow, globose, subglobose to oval or ellipsoid (155–255 × 150–225 µm), and have three walls (an outer, a middle, and an inner wall; OW, MW, and IW) and a bi-lobed, hyaline to subhyaline germination shield.

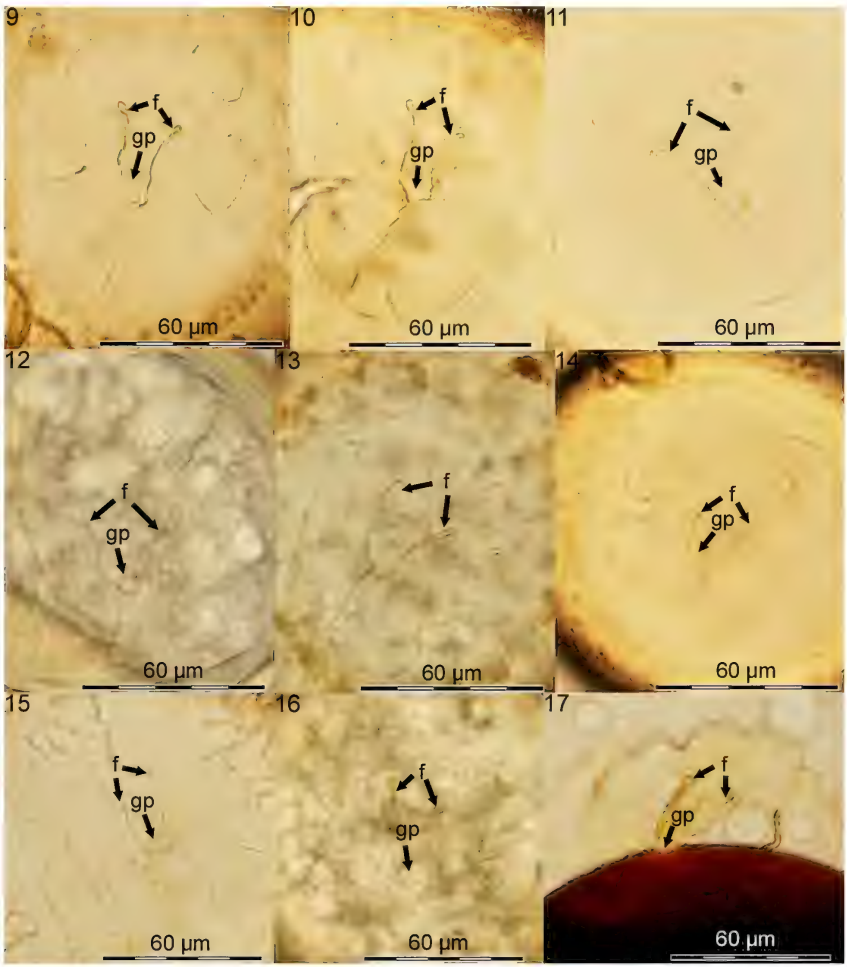
OUTER WALL is 4.0–5.5 µm thick in water, expanding to 7.0–11.0 µm in crushed spores in lactic acid-based mounts when pressure is applied on the cover slide; OW composed of three layers (OWL1–OWL3): the outermost (OWL1) hyaline to subhyaline to light yellow, evanescent to semi-persistent, ~1.0–1.4 µm thick with a papillate surface roughening to which larger debris is often attached, especially while this wall layer is degrading. However, this debris can easily be removed from the spore surface by sonification or slight movements of the spore under the cover slides in liquid or fresh mountants. OWL2 yellowish white to bright yellow, 2.2–3.0 µm thick in H_2O, expanding to 5.2–8.5 µm in lactic acid-based mountants when pressed. OWL3 concolorous with OWL2 or slightly lighter in color, thin, flexible (0.5–1.1 µm thick), and usually difficult to observe as adhering closely to OWL2. OWL2 and OWL3 stain dark yellow in Melzer's reagent. The straight pore channel at the spore base (about 2.8–4.8 µm broad) might be closed by a plug, but often appears to be open.

MIDDLE WALL composed of two hyaline flexible to semi-flexible layers (MWL1, MWL2) that are often difficult to separate. In crushed spores they might show several folds in close vicinity to OW. MWL1 and MWL2 are each about 0.6–1.2 µm thick and may slightly expand in PVLG based mountants.



Figs 1–8. *Scutellospora alterata*, spore morphology: **1–4**. Whitish yellow to light yellow spores formed on sporogenous cells (sc), with three walls (OW, MW, IW) and a single germination shield (gs) on the IW surface. Spore surfaces with papillate roughened (rough) surface often capturing large amounts of debris that can easily be removed through sonification or moving the spores slightly under the cover slides in the first hours after mounting in PVLG. The gs can usually be positioned in planar view through such movements, but often deforms when the oval to ovoid shield shapes (Fig. 3) become irregular and variable (Fig. 4) through pressure. **5–6**. OW triple-layered (OWL1-3), MW bi-layered (MWL1-2), and IW triple-layered (IWL1-3). OW staining dark yellow, and IWL2 purple to deep purple in Melzer's reagent. **7–8**. Papillate roughened spore surface in PVLG and in Melzer's.

Inner wall triple-layered (iwl1- iwl3), bearing a germination shield on the outer surface. Iwl1 hyaline, flexible, 0.4–1.0 µm thin, sometimes difficult to observe; Iwl2 united to finely laminate, 1.4–2.0(–2.7) µm thick; Innermost



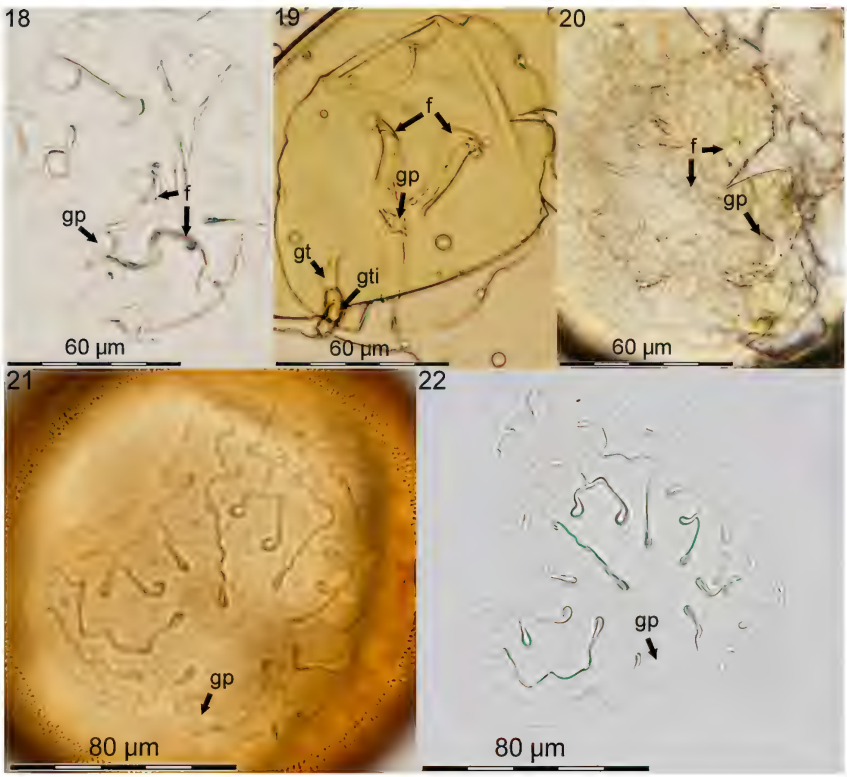
Figs 9–17. *Scutellospora alterata*, variable germination shield morphology. Germ shield is bi-lobed, often oval to ovoid to violin-shaped, bearing a central 'germ pore' (gp) and a 'fork' of two parallel folds (f) separating the lobes. A slight pressure on the spore may cause a few to several 'false' lobes anywhere on the shield periphery. Apparently lobe terminals sometimes do not stop growing close to the central fork and germ pore, but continue recurving laterally (Figs 16–17). However, these processes may also be caused by shield deformation after pressure applied on the spores.

IWL3 flexible, thin (0.4–0.8 µm thick), and often separable from IWL2 when pressure applied on the cover slide and then forming several minute folds. Only IWL2 stains purple to dark purple in Melzer's reagent.

Sporogenous cell globose to elongate, concolorous with or slightly lighter than the spore, 32–50 × 25–41 µm, with 2 wall layers generally visible

(continuous with spore OWL1 and OWL2); OWL1 0.6–1.3 µm thick, evanescent to semi-persistent, roughened as the upper surface of the spore outer wall; OWL2 1.2–2.5 µm thick. Sporogenous hypha bi-layered, 10–26 µm diam tapering to 5–10 µm within 30–120 µm from the sporogenous cell; hyphal wall concolorous with or darker yellow than spore OW, 1.2–2.2 µm tapering to 0.5–1.5 µm. A few (2–5) septa originating from the inner layer might be visible in the hypha.

GERMINATION SHIELD hyaline to subhyaline to (rarely) light yellow, bi-lobed, oval to ovoid (80–100 × 62–80 µm) in uncrushed spores, under pressure becoming irregular (80–130 × 65–125 µm) in crushed or uncrushed spores. Shield has one initial germ pore (GP) at the shield center (in planar view), with two lobes surrounding the two folds of the GP center (= 'fork'). One germ tube initiation (GTIS, 2.2–4.0 µm diam, often difficult to show in photographs) generally positioned towards the end of each lobe. Shield wall thin (0.8–1.6



FIGS 18–22. Deformed bi-lobed germination shields, either on intact or slightly crushed spores or shields completely separated from the spores. **18.** ***Scutellospora alterata***. **19–20.** ***S. calospora*** germination with germ tube (gt) arising from germ tube initiation (gti). **21–22.** ***S. spinosissima*** showing many folds on the shield but no clearly visible gti.

µm), highly flexible, with the shield periphery regularly deforming under light pressure on the cover slide, sometimes even in uncrushed spores, when at any position additional 'false' lobes may arise that are regular to highly irregular in shape but always lacking GTIS.

DISTRIBUTION: Known only from the semi-arid Caatinga biome, in the Municipality of Araripina, in Pernambuco State (NE Brazil).

Spore development

The major stages of spore development could be deduced from clearly identified spores of *S. alterata* found in the bait cultures. First the outer spore wall differentiates one evanescent to semi-persistent outer layer (OWL1), a laminate layer (OWL2), and the adherent thin inner layer (OWL3). The bi-layered middle wall (MW), and later the three-layered inner wall (IW), develop de novo without visible connection with the outer wall. Finally, the germination shield develops on the outer surface of the inner wall as in other *Scutellospora* species (e.g. FIGS 19–22).

Molecular analyses

Phylogenetic analyses of the partial LSU rDNA gene place the fungus within *Scutellospora* in a clade next to *S. calospora*, *S. dipurpurescens*, and *S. spinosissima* (FIG. 23). For the ITS region, the species closest to *S. alterata* were *S. calospora* (92% identity, BLASTn analysis) and *S. spinosissima* (90%). The LSU rDNA sequences were closest to *S. calospora* and *S. dipurpurescens* (both 96% identity). Environmental ITS sequences with closest match to *S. alterata* were found in roots of *Lobelia dortmanna* L. (JN581980, 94% identity; Kohut et al. 2012). The *S. alterata* LSU region was most similar to environmental sequences amplified in roots of *Phellodendron amurense* Rupr. (Heilongjiang, China—EU252109, 96%) and (*Miscanthus sinensis* Andersson (Hokkaido, Japan—AB561124, 96%) and to some sequences obtained from a grassland ecosystem soil (DQ400293, 99%; DQ400277, 97%; Hempel et al. 2007).

Discussion

Scutellospora alterata has two surprising diagnostic features: a) the presence of an evanescent, papillate surface roughening on the spores instead of a smooth surface or persistent surface ornamentation and b) a simple germination shield that may erroneously be interpreted as complex due to the emergence of several 'false' lobes, especially after pressure on the spore. The new fungus can easily be distinguished from all known *Scutellospora* species, since *S. arenicola* (Koske & Halvorson 1990), *S. aurigloba* (Hall 1977, as *Gigaspora aurigloba*), *S. calospora* (Nicolson & Gerdemann 1968, as *Endogone calospora*), *S. dipurpurescens* (Morton & Koske 1988), and *S. tricalypta* (Ferrer & Herrera 1981, as *Gigaspora*

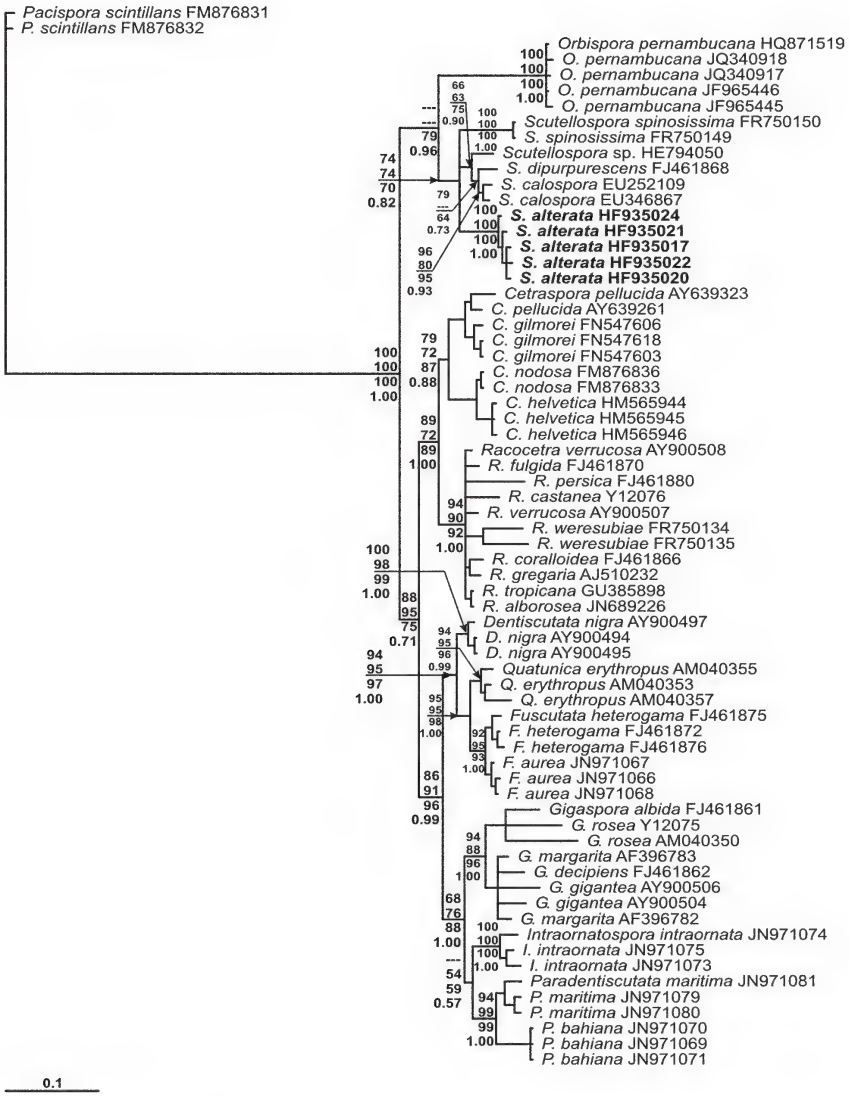


FIG. 23. *Gigasporales*. LSU rDNA-based phylogenetic tree rooted by *Pacispora scintillans*. Sequences are labeled with database accession numbers. Support values (from top) are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. *Scutellospora alterata* sequences are in bold. Only bootstrap values ≥50% are shown. (Consistency Index = 0.53; Retention Index = 0.82).

tricalypta) all have smooth spore surfaces, and *S. crenulata* (Herrera-Peraza et al. 2001), *S. dipapillosa* (Koske & Walker 1985, as *Gigaspora dipapillosa*), and *S. spinosissima* (Walker et al. 1998, Silva et al. 2013) have either double ornamentations or permanent spines on the spore surfaces (Oehl et al. 2008, 2011e).

Roughened spore surfaces occur quite commonly in glomoid species (e.g., *Viscospora viscosa*; Oehl et al. 2011b) but have rarely been found in other glomeromycotan groups, e.g., in *Ambispora granatensis* (Palenzuela et al. 2011). In the *Gigasporales*, *Dentiscutata cerradensis* is known to have papillae on the spore surface (Spain & Miranda 1996) that become invisible in lactic acid-based mountants, whereas the *S. alterata* papillae generally remain visible on spores immersed in lactic acid-based mountants.

Scutellospora species are characterized by hyaline shields that possess only two 'true' lobes, each generally bearing one GTI when mature. The observation that additional, so-called 'false' lobes may arise on germination shield peripheries through pressure on the spore surface is interesting, because the phenomenon has not been reported previously. Certainly, such a feature has never been so obvious and the extent and variability of the shield alterations might be unique for *S. alterata*. Nonetheless, this distortion can also be recognized to a lesser extent in type specimens of *S. dipurpurescens* (OSC), and we also found such characteristics in a *S. calospora* isolate from Germany (FIGS 19–20) and in *S. spinosissima* (FIGS 21–22), which Walker et al. (1998) and Oehl et al. (2008) at first erroneously interpreted as containing more lobes. Indeed, especially in *S. spinosissima,* multiple folds and 'false' lobes can sometimes be seen beneath cover slides when shields are exposed to pressure or lateral movements in liquid lactic acid mountants. Shield morphology has not yet been analyzed sufficiently in the *Scutellosporaceae* (including *Orbispora*), and we feel that such investigations should be carried out soon.

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A new *Drechslerella* species from Hainan, China

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Abstract — *Drechslerella hainanensis* sp. nov., isolated from soil sampled from Wuzhi Mountain, Hainan Province, China, is described. The new species, placed in *Drechslerella* because it forms constricting rings in the presence of nematodes, is characterized by unbranched conidiophores and 0–2-septate subcylindric–ellipsoidal macroconidia. The morphological and phylogenetic differences between *D. hainanensis* and similar species are discussed.

Key words — ITS, nematode trapping fungi, orbiliaceous, phylogenetic placement

Introduction

Scholler et al. provided evidence by detailed molecular analyses to propose new generic concepts for orbiliaceous nematode-trapping fungi based on the shape of trapping device (Hagedorn & Scholler 1999, Scholler et al. 1999). Li et al. (2005) also redefined the systematic classification of nematode-trapping fungi based on phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA, and β-tubulin genes and confirmed that it is credible to classify nematode-trapping fungi based on the shapes of their trapping devices. According to the present systematics of nematode-trapping fungi, *Drechslerella* Subram. is characterized by three-celled constricting ring traps.

In our survey of nematode-trapping fungi from Hainan Province, a new species was isolated from soil, which we referred to *Drechslerella* based on its formation of three-celled constricting ring traps. In this paper, we describe and illustrate the new species and discuss its phylogenetic placement determined by ITS sequence analyses.

[#] Jian-Ying Li & Wen-Yun Qian contributed equally to this work

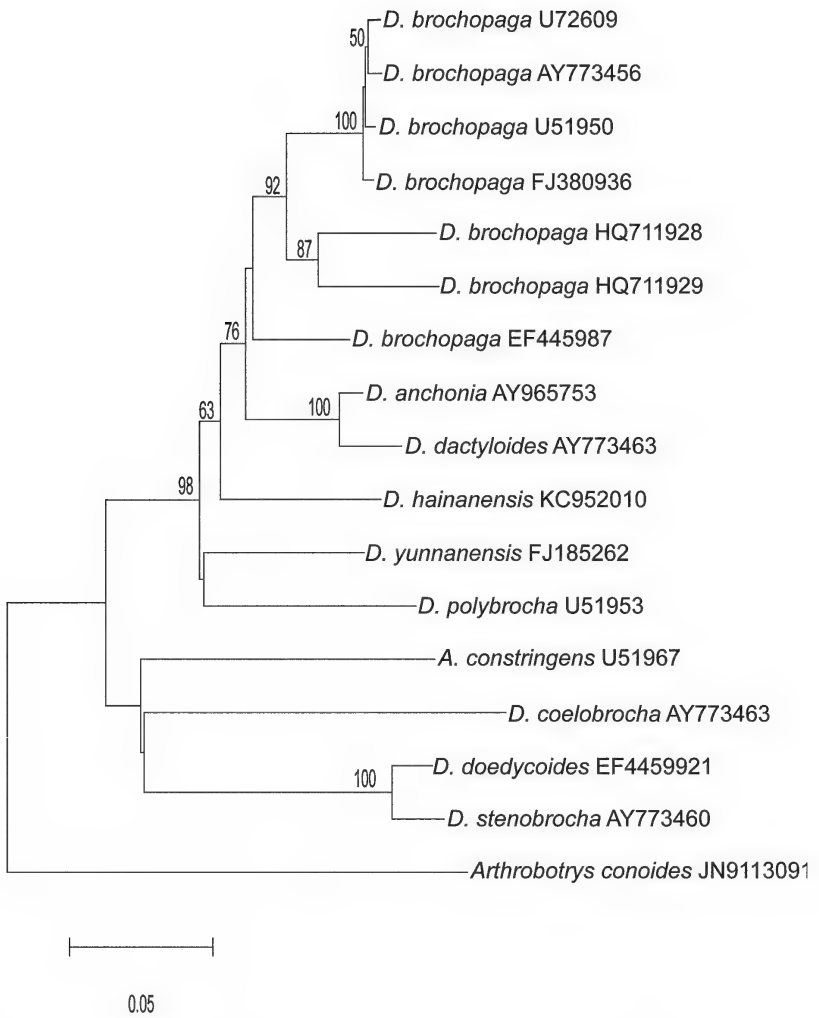

Plate 1. Phylogenetic tree of *Drechslerella hainanensis* and nine other *Drechslerella* species, including *Arthrobotrys constringens* (= *D. effusa*), using the neighbor joining method based on ITS region sequence data. *Arthrobotrys conoides* was used as outgroup. Bootstrap values <50% are not shown.

Materials & methods

Collection of samples, isolation and characterization

Soil samples (30 g) were collected from the forest in Wuzhi Mountain, Hainan Province, China. Each sample was placed in zip-locked plastic bags and labeled.

Samples of 0.5–1g soil were sprinkled onto CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) inoculated with sterile *Paragrellus redivius* (free-living nematode) and incubated at 25°C, following methods described by Duddington (1955) and Wyborn et al. (1969). After one month, samples were examined using a dissecting microscope. Single spores were isolated with a sterilized toothpick and were cultivated on CMA at 25°C, maintained on the same medium as agar slants and stored at 4°C. Morphological observations were made from CMA after incubation at 28°C for one week.

Specimens and cultures were conserved in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources (YMF), Yunnan University, Kunming, Yunnan, P.R. China.

DNA extraction, PCR and sequencing

Total DNA was isolated from fresh mycelium as described by Turner et al. (1997). Primer pairs ITS4 and ITS5 (White et al. 1990) were used to amplify the complete ITS. PCR amplification parameters followed Yu et al. (2007). The PCR products were purified with a commercial Kit (Bioteke Biotechnology Co., Ltd., China) and sequenced on both strands with the same primers that were used for amplification with the aid of a LI-COR 4000L automatic sequencing system, using cycle sequencing with the ThermoSequenase-kit as described by Kindermann et al. (1998).

Phylogenetic analysis

Using the new ITS sequence from our culture and the 15 available GenBank ITS sequences from nine other *Drechslerella* species, we performed cladistic analyses with MEGA version 4.1 using the neighbor-joining method. The neighbor-joining tree was constructed with Kimura 2-parameter model, including transitions and transversions with pairwise gap deletion.

Results

A neighbor-joining tree (Plate 1) was generated from ITS sequences from *D. hainanensis* and nine other *Drechslerella* species. *Arthrobotrys conoides* Drechsler, a fungus trapping by means of adhesive three-dimensional networks, was selected as outgroup. The phylogenetic tree shows that *D. hainanensis* clusters as a species separate from *D. doedycoides* (Drechsler) M. Scholler et al., *D. effusa* (Jarow.) M. Scholler et al., and *D. polybrocha* (Drechsler) M. Scholler et al.

Taxonomy

Drechslerella hainanensis Jian Y. Li & Z.F. Yu, **sp. nov.** Plate 2

MycoBank MB 804093

Differs from *Drechslerella effusa* by its production of only a single macroconidium at the conidiophore apex.

Type: PR China, Hainan Province, Wuzhi Mountain, Atuoling Provincial Park, 18°48′N 109°34′E, elev. 514 m, isolated from forest soil, Dec. 2010, Y. L. Bai (**Holotype**, YMF 1.03963; ex-type culture, YMF 1.036931).

Etymology: *hainanensis* refers to the province in which the species was found.

Mycelium slow-growing, colonies hyaline to white, reaching ≤25 mm diam. after 10 days on CMA at 25°C. Vegetative hyphae hyaline, septate, 1.2–4.9 µm wide. Aerial mycelium sparse, hyaline, septate, branched. Macroconidiophores hyaline, septate, erect, unbranched, 98–109 × 3.5–5.0 µm, each tip bearing one conidium. Macroconidia hyaline, straight, subcylindric-ellipsoidal, 32.5–43 µm long, 17.0–25 µm at the broadest part, (0–)1–2 septate (0 in 18.3%; 1 in 40.2%; 2 in 41.5%; n = 80). Microconidia frequently observed. Microconidiophores hyaline, septate, erect, unbranched, 40–80 × 2.2–3.3 µm, sometimes narrower near apex, apices with 2–4 4.5–6.0 long denticles, each bearing one conidium. Microconidia hyaline, straight, elongate ellipsoid-clavate, rounded at the apex, 18.2–22.8 × 4.2–5.3 µm, (0–)1 septate. Trapping nematodes by means of spontaneously formed, stalked, three-celled constricting rings with triangular inner thickenings at the septa. At non-constricted points, outer diameter = 22–35.5 µm, inner diameter = 9.0–15.5 µm.

Discussion

Drechslerella heterospora (Drechsler) M. Scholler et al., *D. stenobrocha* (Drechsler) M. Scholler et al., and *D. effusa* resemble *D. hainanensis* in their cylindric-ellipsoid shaped macroconidia. *Drechslerella heterospora* differs from *D. hainensis* by its distinctly narrower microconidia and *D. stenobrocha* differs from *D. hainensis* by its distinctly narrower macroconidia. *Drechslerella effusa* fits quite well with *D. hainanensis* in macroconidial size and number of septa but differs in producing up to 12 or more macroconidia near the conidiophore apex, whereas the macroconidia of *D. hainanensis* always grow singly at the tip. Unlike *D. hainanensis*, *D. doedycoides* macroconidia are broadly ellipsoid-fusoid with narrowed ends and 2(–3)-septate, while those of *D. polybrocha* have only one sub-basal septum.

Our ITS rDNA phylogenetic tree supports *D. hainanensis* as separate from *D. doedycoides*, *Arthrobotrys constringens* (= *D. effusa*), *D. polybrocha*, and *D. stenobrocha*. We included a sequence from the type of *Arthrobotrys constringens* Dowsett et al. because no sequence was available from the *D. effusa* type; Oorschot (1985) treated these two species as synonymous, although they differ somewhat in microconidial size. Although *D. hainanensis* is very close to the clade formed by *D. brochopaga* (Drechsler) M. Scholler et al., *D. anchonia* (Drechsler) M. Scholler et al., and *D. dactyloides* (Drechsler) M. Scholler et al., in these three species the conidiophore apex bears more than one conidium, thus forming a radiating capitate arrangement, and their conidial shape and septation also differ from those in *D. hainanensis*.

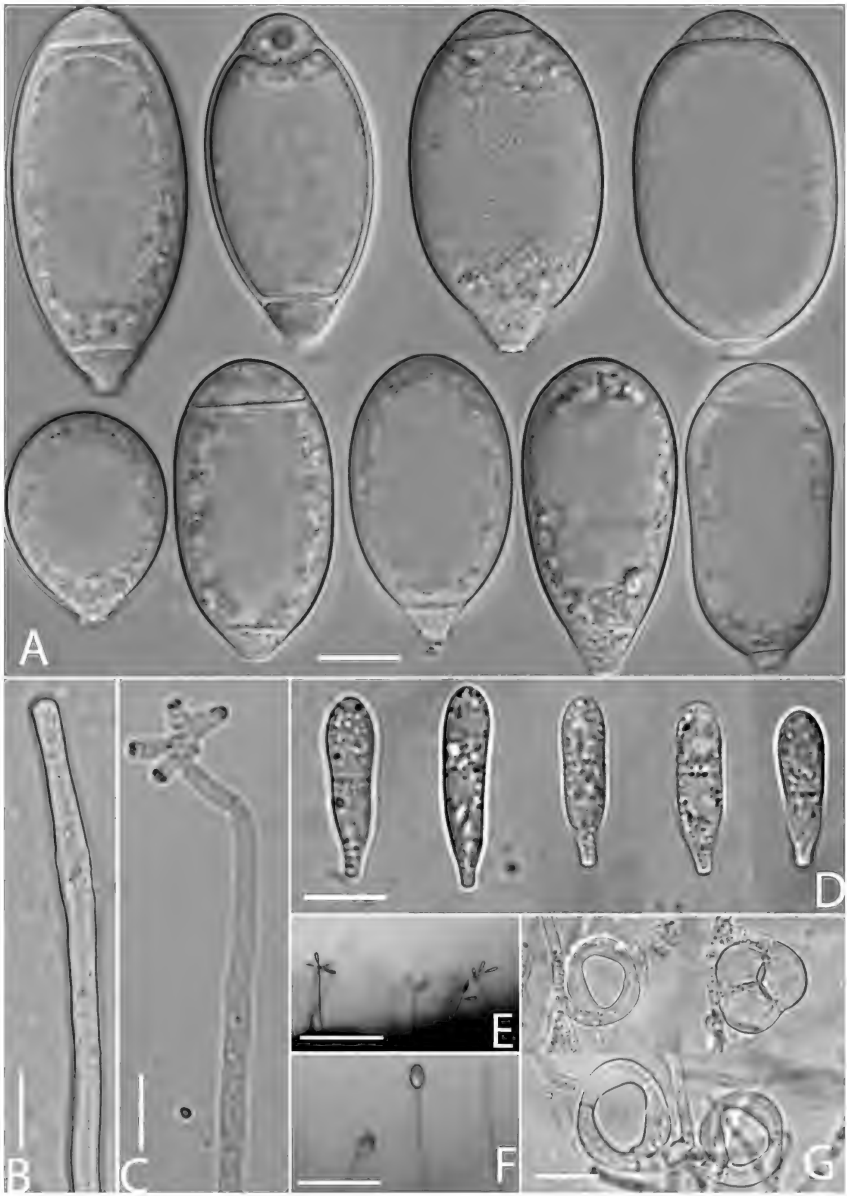

Plate 2. *Drechslerella hainanensis* (**Holotype**, YMF 1.03963): A. Macroconidia; B, C. Macroconidiophore; D. Microconidia; E. Microconidiophores and microconidia; F. Macroconidiophore and macroconidia; G. Constricting rings. Scale bars: A–D = 10 μm; E, F = 50 μm; G = 20 μm.

Acknowledgements

This work was jointly financed by National Basic Research Program of China ('973'Program: 2013CB127503) and National Natural Science Foundation Program of PR China (31060008, 31160008, 31260007, 31360130), Grants from the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI020). China National Tobacco Corporation (grant no. 110201002023) and Yunnan Branch of China Tobacco Industrial Corporation (grant no. 2010YN17). We are very grateful to Dr. D. M. Hu and Prof. H.-O. Baral for critically reviewing the manuscript and providing precious suggestions on this paper.

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New reports of cyperaceous rust fungi from Pakistan

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Abstract —*Puccinia minuta* is reported as new record, and the telial stage of *P. caricis* is reported for the first time from Pakistan.

Key words — *Cyperaceae*, sedges, *Uredinales*

Introduction

This paper is a continuation of the exploration of rust fungi on *Poaceae* and *Cyperaceae* from Pakistan. Previously, about 21 rust species have been reported on 15 hosts of *Cyperaceae* from Pakistan (Ahmad et al. 1997; Afshan & Khalid 2008; 2009; Afshan et al. 2009; Saba & Khalid 2011). In the present investigation, rusted specimens of *Cyperaceae* were collected from different localities in Pakistan. Among these, one rust is a new record for the country, and the telial stage of another is reported for the first time in Pakistan.

Materials & methods

Rust infected plants were collected along with their inflorescences. These collections as well as herbarium specimens from were studied morphologically and free hand sections and scrape mounts were taken from infected plant materials. The plants were photographed and infected portions were observed under a stereomicroscope. Twenty spores of each spore state were examined under a microscope (Nikon YS 100); paraphyses and spores were measured through a Zeiss Eyepiece Screw micrometer. Sections, paraphyses, and spores were photographed using a Digiporo-Labomed. Illustrations of spores and paraphyses were made under a camera lucida (Ernst Leitz Wetzlar Germany).

Species recorded

Puccinia minuta Dietel, Cornell Univ. Sci. Bull. 3(1): 19 (1897) Fig. 1

Spermogonia and aecia not found. Uredinia adaxial, naked, light brown in color, 0.2–0.3 mm long. Urediniospores globose to subglobose, light brown to

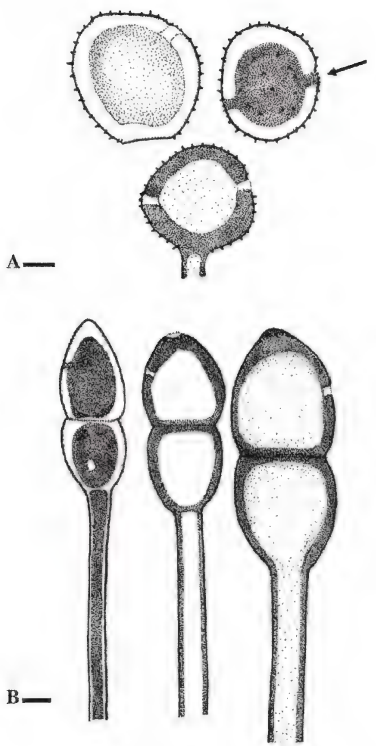

Fig. 1: *Puccinia minuta.*
A. Urediniospores showing germ pores (arrow); B. Teliospores.
Scale bars: A = 14μm; B = 12 μm.

hyaline, 23–29 × 24–30(–38) μm; wall 2–4 μm thick, echinulate; germ pores up to 4, equatorial. Telia amphigenous, subepidermal, brown, 0.1–0.4 × 0.1–0.3 mm. Teliospores amphigenous, oblong, brown to chestnut brown, 2-celled, (9–)14–24(–27) × (26–)42–56 μm, rounded above, narrowed below, apex 5–9 μm thick; wall 2–5 μm thick; pedicel long, hyaline, 5–9 × 30–66(–92) μm.

Material examined: **PAKISTAN**, **Khyber-Pakhtunkhawah** (KPK), Ghora gali, alt. 1950 m a.s.l., on *Carex* sp., stages II + III, April 2011, AM #7 (LAH 1135).

Comments: *Puccinia bolleyana* Sacc., *P. caricina* DC., *P. caricis-kouriyamensis* Morim., *P. caricis-pocilliformis* Morim., *P. dioicae* Magnus, *P. extensicola* var. *linosyridis-caricis* (E. Fisch.) Zwetko, *P. pakistani* S. Ahmad, *P. subepidermalis* Afshan et al., and *P. urticata* F. Kern have previously been reported on *Carex* spp. from Pakistan (Ahmad 1960, 1969; Ono 1992; Ono & Kakishima 1992; Afshan & Khalid 2009; Afshan et al. 2011; Saba & Khalid 2011). *Puccinia minuta* is a new record for the country.

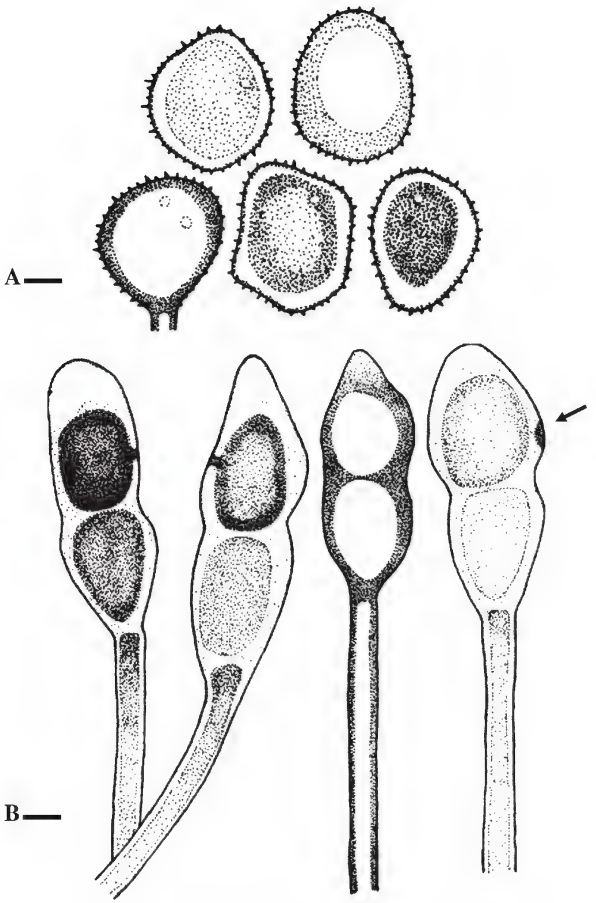

Fig. 2: *Puccinia caricis.*
A. Urediniospores showing germ pores (arrow); B. Teliospores.
Scale bars: A = 10 µm; B = 13 µm.

Puccinia caricis Rebent., Prodr. Fl. Neomarch.: 356 (1804) Fig. 2

Spermogonia and aecia not found. Uredinia amphigenous, light brown, scattered, covered by epidermis, oblong, 0.1–0.2 × 0.07–0.1 mm. Urediniospores globose to subglobose, 14–19 × 18–23 µm; wall 2–3 µm thick, echinulate; germ pores up to 8, scattered. Telia hypophyllous, naked, black, 0.1–0.2 mm. Telia hypophyllous, naked, black, 0.1–0.2 mm. Teliospores clavate or oblong to clavate, chestnut-brown to hyaline, 16–26 × 47–68 µm; apex conical, 8–10 µm thick; 1.5–2 µm thick from sides; pedicels hyaline, persistent, up to 50 µm long.

Material examined: **PAKISTAN, Khyber-Pakhtunkhawah** (KPK), Ghora gali, alt. 1950 m a.s.l., on *Carex* sp., stage III, April, 2011, AM # 61 (LAH 1136).

Comments: The aecial stage of *Puccinia caricis* was previously reported from Patriata in Pakistan on leaves of *Urtica dioica* L. (Ahmad 1956a,b). The telial stage is reported here for the first time from the country.

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Scleroderma yunnanense, a new species from South China

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Abstract — *Scleroderma yunnanense* sp. nov. is described from Yunnan, China. Previously misidentified as *Scleroderma citrinum,* the new species is diagnosed by its echinulate-spiny spores and thick peridium. Molecular analysis supports erection of this new species. Perhaps unique for the genus, *S. yunnanense* is edible and considered a delicacy in Yunnan.

Key words — earth ball, gasteromycete, taxonomy

Introduction

Scleroderma (*Sclerodermataceae*, *Boletales*) has about 25 recognized species (Guzman 1970; Sims et al. 1995; Kirk et al. 2008), of which 10 are found in China (Liu 2005). The genus is widespread from the tropical to boreal regions and common in forests, parks, and farmland. Most mushroom books and scientific papers state that *Scleroderma* species are suspected, undesirable, inedible, or poisonous (Arora 1986; Bresinsky & Besl 1990; Mao 1998; Pacioni 1981; Stevenson & Benjamin 1962). Bresinsky & Besl (1990) state that consumption of raw *Scleroderma cepa* Pers. leads to severe abdominal pain, stomach cramp, and paralysis, while in New Zealand ingestion of an unknown *Scleroderma* species cooked in a casserole resulted in anaphylactic shock (I.R. Hall, pers. comm.). In stark contrast, the authors have found an edible *Scleroderma* species that is extensively collected and sold at local mushroom markets in Yunnan, China, without any reports of adverse affects on humans. The authors, who have eaten this mushroom on a number of occasions, have found it to be not just edible but choice and delicious. This species is commonly found growing in the tropical and warm-subtropical parts of Yunnan Province. Previously this mushroom was recorded from China as *S. citrinum* Pers. (Wang et al. 2004).

However, after re-examining recent collections both morphologically and molecularly we determined that this species has been incorrectly identified and warrants separation as a new species.

Materials & methods

The macroscopic and microscopic characters were described based on fresh and dried specimens collected from several locations in Yunnan, China. Sections were made with a freezing microtome (Jinhua Yidi Medical Equipment Factory, Zhejiang, China) and mounted in water and then lactophenol solution. Dry specimens were mounted in 5% KOH solution and examined under a Nikon E400 microscope. Spore samples were vacuum-dried, sputter-coated with gold, and examined with a FEI QUANTA200 Environmental Scanning Electron Microscope at Yunnan University.

The holotype and other examined specimens are deposited in KUN-HKAS, Kunming, Yunnan, China, except for one specimen held in the Herbarium of the Research Institute

Table 1. *Phlebopus* and *Scleroderma* collections included in the molecular analysis.

Species	Voucher	Origin	Genbank *
P. portentosus	H-01-3-1	China	GQ253574
S. areolatum	JMP0080	USA	EU819438
	RT00036	USA	EU819518
	F: PGK193	USA	GQ166910
S. aurantium	Strain 8-5	China	HM237174
S. bovista	K80S09	New Zealand	GQ267487
	K(M)105588	England	EU784409
	RT00034	USA	EU819517
S. cepa	JMP0081	USA	EU819439
S. citrinum	F:PRL5772	USA	GQ166907
	K(M)17485	England	EU784413
	CITSCL1	USA	FM213344
	K(M)53906	England	EU784414
	—	USA	FJ824090
S. polyrhizum	Strain 11-3	China	HM237173
S. sinnamariense	SINSCL9	Thailand	FM213364
	SINSCL8	Thailand	FM213363
	SINSCL6 (SCLD1)	Thailand	FM213361
	SINSCL7	Thailand	FM213362
	SINSCL2 (SCLP3)	Thailand	FM213357
S. verrucosum	K(M)30670	England	EU784415
S. yunnanense	KUN-HKAS 79633A	China	**JQ639040**
	KUN-HKAS 79633B	China	**JQ639041**
	KUN-HKAS 79633C	China	**JQ639042**
	KUN-HKAS 79633D	China	**JQ639043**
	KUN-HKAS 79664A	China	**JQ639044**
	KUN-HKAS 79664B	China	**JQ639045**
	KUN-HKAS 79665	China	**JQ639046**
	IFRD 414-012	China	FJ687275

*New sequences are presented in bold font.

of Resource Insects of Chinese Academy of Forestry (IFRD), Kunming, Yunnan, China.

Tissue samples were excised from the inner part of fresh basidiomata to avoid contamination. DNA was extracted from each sample with the E.Z.N.A. fungi DNA miniprep kit (Omega Biotech, GA, and USA). The ITS region was amplified with TransTaq™ DNA Polymerase High Fidelity (TransGen Biotech, China). Primer pairs ITS4/ITS5 were used to amplify the ITS region of the ribosomal RNA gene (White et al. 1990). PCR parameters were as follows: initial denaturation for 5 min at 95 °C followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were electrophoresed on 1.2% agarose gels with 1× TBE buffer. After purification with a Gel Extraction & PCR Purification Combo Kit (BioTeke, China), the PCR products were ligated into a pMD-18T vector (TakaRa, Japan) for DNA sequencing. Both strands were sequenced using the vector specific primers T7 and M13F (BGI Company). Sequences of seven samples were submitted to GenBank (Table 1). Closely related sequences revealed by BLASTN were obtained from the GenBank DNA database (http://www.ncbi.nlm.nih.gov/entrez/). A total of 29 sequences were used for phylogenetic analyses (Table 1).

A multiple sequence alignment was performed using the ClustalX programme (Thompson et al. 1998). Phylogenies were constructed using the MEGA 4.0 software package (Kumar et al. 2008). Neighbor-Joining (NJ) trees were constructed using the Kimura-2-parameter distance model and Gamma distribution parameter 0.55. Tree robustness was assessed with a 1000-pseudoreplicate Bootstrap analysis (Felsenstein 1985). Branches supported by >50% of bootstrap replicates are indicated on the tree. *Phlebopus portentosus* (GQ253574) was used as the outgroup.

Taxonomy

Scleroderma yunnanense Y. Wang, **sp. nov.** Figs. 1–12

MycoBank MB 805073

Differs from *Scleroderma cepa* by having clamp-connections and from *S. sinnamariense* in its thicker peridium with a hyaline inner surface and its non-reticulate basidiospore ornamentation of dense narrow pyramidal warts.

Type: China, Yunnan Province, Pure, 22°20'N 110°59'E, under *Pinus kesiya* var. *langbianensis*, 15 Sept. 2011: K.P. Ji S1101 (**Holotype**, KUN-HKAS 79633; GenBank JQ639040–JQ639043).

Etymology: *yunnanense* refers to the type locality.

Basidiomata subglobose to globose, 2.5–5.0(–7.0) cm in diameter, with scales, dirty whitish to yellowish, the base attached to the substrate via a tuft of mycelium and rhizomorphs, sometimes aggregated into a pseudostipe, ≤2 cm long, conspicuously covered with well-developed rhizomorphs composed of hyaline hyphae, usually 2.0–3.0(–5) μm in diam., with emanating right branching hyphae and numerous clamp-connections and with some hyphae with adherent crystalline structure. Peridium 2–7 mm thick, two-layered, the outer layer ≤500 μm thick, composed of thin and thick yellowish hyphae

towards the peridial surface, 1.5–2.0(–5) µm in diam.; the inner layer ≤6500 µm thick, composed of hyaline interwoven hyphae, 5.0–7.5 µm diam., clamp-connections rare. KOH on the surface of peridium light reddish and no reaction on peridial cross-section. GLEBA when young firm, white to whitish, and with numerous white capillitia, becoming grayish to purplish then purplish brown and finally powdery, composed of hyaline hyphae, 1.5–2.0 µm in diam., with a few dark, thick hyphae ≤3 µm in diam. BASIDIOSPORES globose to subglobose, (7.0–) 8.4 × 8.0(–9.5) µm including ornamentation, covered with dense narrow pyramidal warts, 1–1.2 µm long and 0.4–0.6(–8) µm thick at the base. ODOR mushroomy; TASTE sweet.

PHYLOGENETIC ANALYSIS — ITS-rDNA sequence analysis groups all *S. yunnanense* collections together with high (99%) bootstrap value and separate from the *S. citrinum* collections, which also group together with high (97%) bootstrap value (FIG. 13).

ECOLOGY & DISTRIBUTION — Sub-hypogeous, solitary or in small groups under *Pinus kesiya* var. *langbianensis* and *Betula alnoides* and forming ectomycorrhizae with one or both of these hosts, alt. 880–1300 m, fruiting from June to October in Yunnan, China. Known only from sub-tropical and tropical regions of Yunnan, China.

ADDITIONAL SPECIMENS EXAMINED: **CHINA, YUNNAN PROVINCE, Pure** — 22°20'N 110°59'E, under *Pinus kesiya* var. *langbianensis,* 15 Sept. 2011: K.P. Ji S1102 (KUN-HKAS 79664; GenBank JQ639044, JQ639045); under *Betula alnoides,* 15 Sept. 2011: K.P. Ji S1103 (KUN-HKAS 79665; GenBank JQ639046); **Puwen** — 22°30'N 101°03'E, under *Pinus kesiya* var. *langbianensis,* 24.Sep.2011: Ji S1104 (KUN-HKAS 79666), Ji S1105 (KUN-HKAS 79667), Ji S1106 (KUN-HKAS 79668); **Yiliang** — 24°92'N 103°14'E, 12.Sep.2007: T. Ma, YL007 (IFRD 414-012, as "*Scleroderma citrinum,*" GenBank FJ687275); **Wuiding** — 25°55'N 102°36'E, 12.Aug.1998: X.H. Wang 742, KUN-HKAS 35824, as "*Scleroderma citrinum*").

COMMENTS —The discovery of an edible *Scleroderma* species in a genus long considered toxic (or at least highly suspect) underlines the difficulties in generalizing on the edibility or toxicity of mushrooms. In the Yunnan area where *S. yunnanense* is widely consumed, there is presumably sufficient general knowledge to prevent the eating of inedible or toxic lookalikes. However, the

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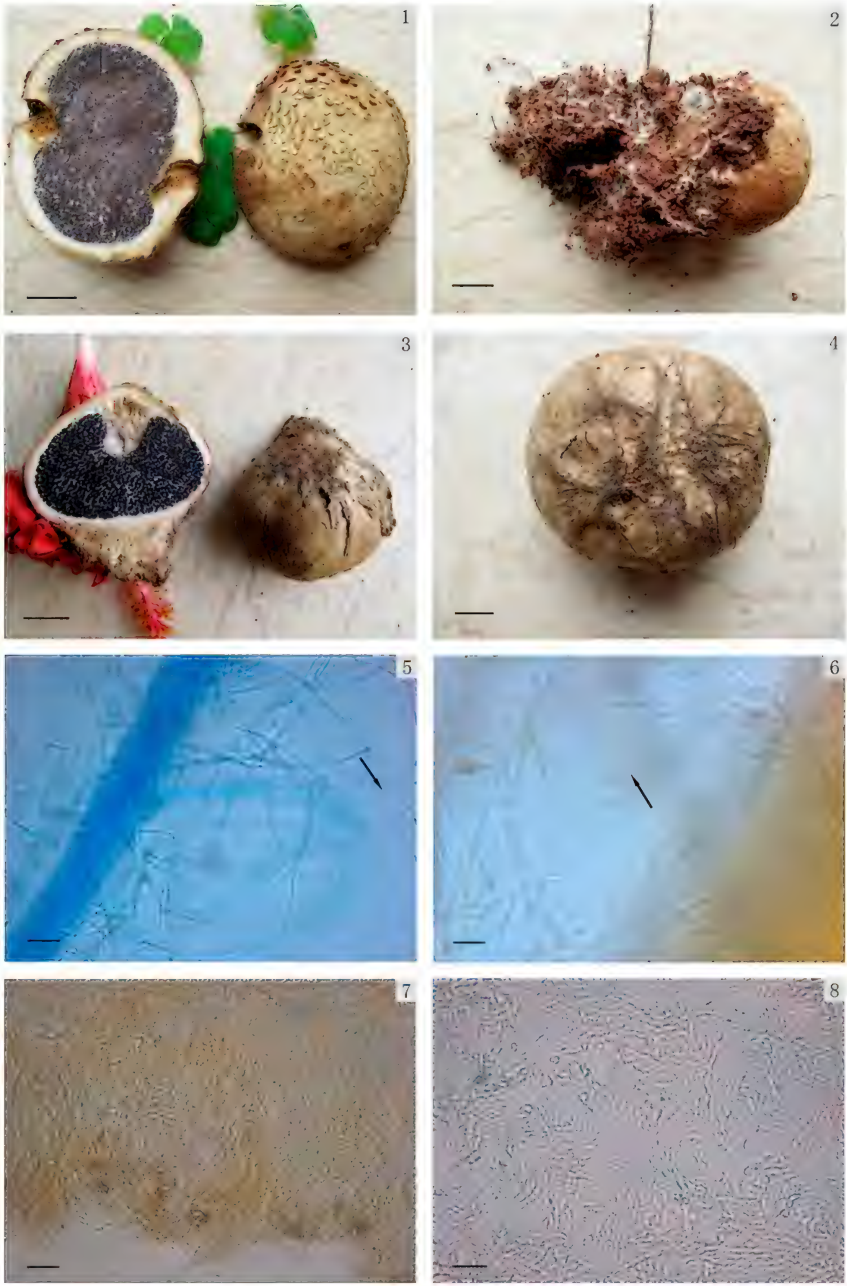
FIGS 1–8. *Scleroderma yunnanense.* 1: Sectioned fruiting body, showing scales and gleba. 2: Fruiting body, showing dense rhizomorphs in the soil. 3: Fruiting body with rhizomorphs, sectioned to show the pseudostipe. 4: Rhizomorphs on the fruiting body surface. 5: Rhizomorphs and emanating hyphae, showing right-angled hyphal branching (arrow) and clamp-connections. 6: Rhizomorphs and emanating hyphae, showing crystalline structure on the hyphae (arrow). 7: Outer peridial layer with vertically arranged hyphae. 8: Inner peridial layer of interwoven hyphae. Scale bars: 1, 3 = 1.5 cm; 2, 4 = 1 cm; 5 = 40 µm; 6 = 25 µm; 7, 8 = 50 µm.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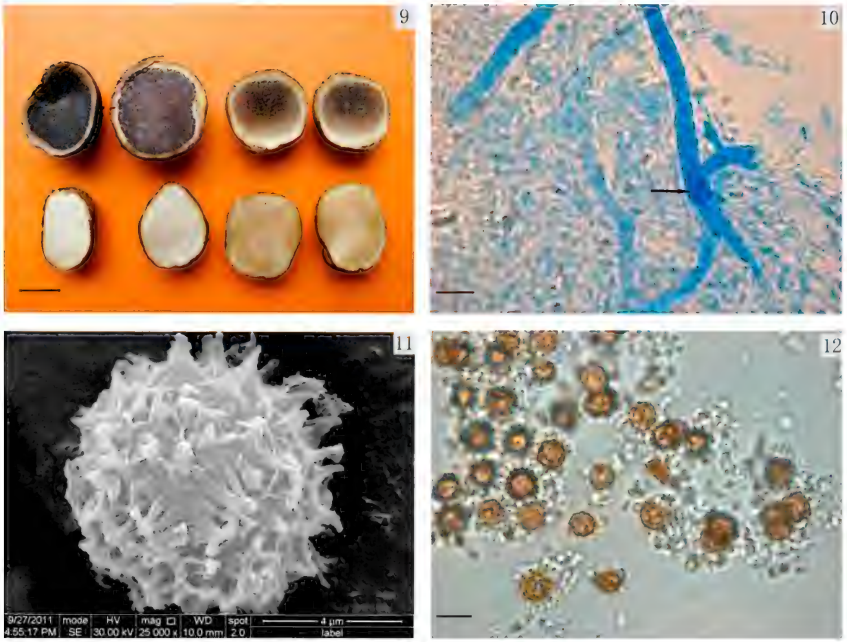

Figs 9–12. *Scleroderma yunnanense*. 9: Basidiomata showing various stages of maturity. 10: Gleba sporogenous tissue, showing thick hyphae (arrow). 11: Basidiospore (SEM). 12: Basidiospores. Scale bars: 9 = 2 cm; 10 = 2.5 µm; 11 = 4 µm; 12 = 10 µm.

likely publicity surrounding the edibility of *S. yunnanense* might now persuade some people to try eating potential lookalikes outside of the natural range of this species. Fortunately, the misidentification of *S. yunnanense* as *S. citrinum* Pers. (a toxic species) should be lessened based on the accurate morphological information that we present.

Morphologically *Scleroderma yunnanense* is similar to *S. cepa* Pers. in having spiny spores and thick peridium. However, it differs in having clamp-connections (Fig. 8). The ITS-rDNA sequence analysis shows that *S. yunnanense* is closely related to *S. sinnamariense* Mont. (Fig. 13). However, *S. sinnamariense* has a thinner (<1 mm) peridium, a bright sulfur yellow inner peridial surface, and partially reticulate spores, all of which clearly differ in *S. yunnanense*.

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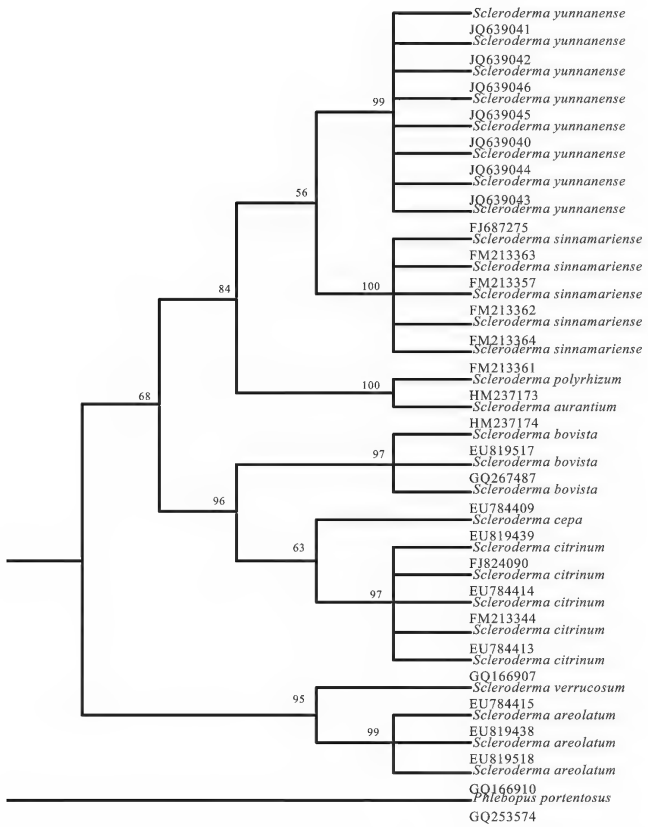

FIG. 13. ITS-based neighbor-joining phylogenetic tree of *Scleroderma* species. Sequences are labeled with their GenBank accession numbers (see TABLE 1). Bootstrap support values (1000 replications) >50% are shown on the nodes.

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New records of microfungi from Turkey (Bartin Province)

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Abstract — *Cheiromyces stellatus, Coniothyrium ruscicola, Metasphaeria errabunda, Mycosphaerella winteri, Nectria viridescens, Neosetophoma samararum, Oletheriostrigula papulosa* and *Physalospora eriostega* are recorded for the first time for the Turkish mycobiota, and are described and illustrated based on Turkish specimens. These represent the first records from Turkey of the genera *Cheiromyces, Neosetophoma*, and *Oletheriostrigula*.

Key words — *Ascomycota*, fungal diversity, anamorph, teleomorph

Introduction

Bartin province (41°53' N 32°45' E), located on the northwest coast of the Black Sea region, is characterized by its temperate marine ("Black Sea") climate with hot summers and cool winters (Akman 1990). Around Bartin (Karabuk-Zonguldak, Bartin) the Euro-Siberian vegetation type is common and dominant but mixed with a considerable number of Mediterranean species. Mediterranean maquis communities, composed of such species as *Carpinus betulus* L., *Fagus orientalis* Lipsky, and *Castanea sativa* Miller, occur near the Bartin river, although they are generally spread throughout the coastal region. The vegetation and humid climate create favourable conditions for development of fungi.

Although the Bartin forest ecosystem vegetation has been sufficiently examined, but the microfungi of this region are little known. During a recent field study, we collected more than one hundred plant specimens showing symptoms of microfungal infection. Among these specimens, 45 species from 35 genera of *Ascomycota* and *Basidiomycota* were identified. Three ascomycete genera and eight species represent new records for the mycobiota of Turkey. The eight microfungi are described here.

Materials & methods

Fungal specimens were collected in September 2012 during a special research field trip in Peliken forest ecosystems of Bartin Province. The specimens were cut by hand into sections using a razor blade and microscopically examined using Leica DM-E compound microscope. The fungi were identified using the relevant literature (Saccardo 1882; Saccardo & Saccardo 1905; Grove 1935; Tomilin 1979; Dennis 1981; Byzova & Vasyagina 1981; Sutton 1985, 2004; Huhndorf & Harris 1996; Mel'nik 2000; Gruyter et al. 2010). Host plants were identified using the "Flora of Turkey and East Aegean Islands" (Davis 1965–85). Species names follow Index Fungorum (2013), and author names follow Kirk et al. (2008). All specimens are deposited in the Herbarium of Bartin University, Bartin, Turkey (HBU); collection numbers of Elşad Hüseyin are prefixed with EH. Genera new for Turkey are marked with an asterisk (*).

Results

Coniothyrium ruscicola D. Sacc., Mycoth. Ital.: no. 1694. 1913. Fig. 1

Conidiomata black, scattered, immersed, opening by a rounded, simple, distinct ostiole 10–15 µm in diam., globose, 100–120 µm diam. Conidia unicellular, yellowish-green, biguttulate, straight or slightly curved, narrowly ellipsoid or cylindrical with rounded ends, 5–9 × 2.5–3(–3.5) µm.

Causing roundish, ellipsoidal, brownish, spots on cladodes, 1–5 mm in diam., with a reddish brown border.

Material examined: **TURKEY, Bartin Province**, Peliken area, 80 m a. s. l., on living cladodes of *Ruscus hypoglossum* L., 16.IX.2012. E. Hüseyin EH-B 19 (HBU).

Notes: Conidia of the Turkish material are similar in size to those described by Grove (1935, as *Phyllosticta ruscicola:* 7–8 × 3.5 µm).

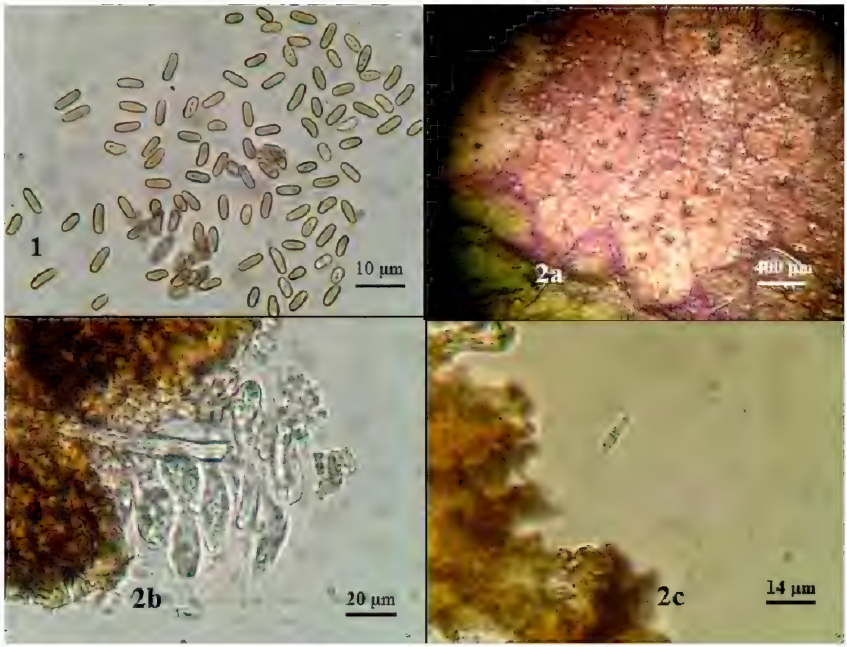
Mycosphaerella winteri (Pass.) Tomilin, Nov. Sist. Niz. Rast. 5: 168. 1968. Fig. 2

Ascomata epiphyllous, scattered, immersed or partly erumpent, dark brown or almost black, sphaerical, 60–90 µm diam., with an ostiole 15–20 µm diam. Asci 8-spored, fasciculate, straight or slightly curved, sessile or short-stalked, clavate or almost cylindrical, 35–45 × 8–10(–11) µm. Ascospores distichous, straight or slightly curved, hyaline, uniseptate, clavate or almost fusiform, 12–15 × 2.5–3.5 am.

On leaves, causing rounded to irregular leaf spots, 4–13(–15) mm diam., confluent, dark brown, with pale brown center.

Material examined: **TURKEY, Bartin Province**, Peliken area, 80 m a.s.l., on living leaves of *Rubus sanctus* Schreb., 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 22 (HBU).

Notes: Ascomata and asci of the Turkish material are similar in size to those presented by Tomilin (1979: ascomata 80–100 µm diam., asci 35–40 × 8–10 µm).



Figs. 1–2. (1) ***Coniothyrium ruscicola:*** conidia. (2) ***Mycosphaerella winteri:*** a. ascomata; b. asci; c. ascospore.

Metasphaeria errabunda Feltgen, Vorstud Pilzfl. Luxemb., Nachtr. 3: 235. 1903. Fig. 3

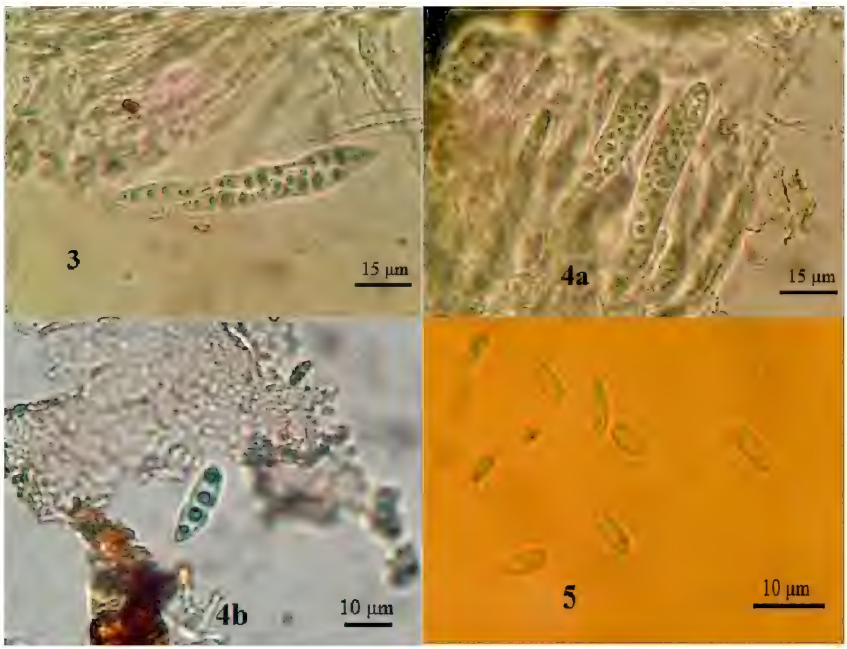
Ascomata scattered or grouped, black, globose-depressed, subimmersed or superficial, 85–160 µm diam. Asci 8-spored, fasciculate, short-stalked, paraphysate, clavate-fusiform, 77–95 × 10–12(–15) µm. Ascospores distichous, hyaline, 3-septate, constricted at the middle septum, 4-guttulate, slightly curved, fusiform, 20–25 × 4–6 µm.

Material examined: **TURKEY, Bartin Province**, Peliken area, 70 m a. s. l., on thin dead branches of *Ilex aquifolium* L., 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 29 (HBU).

Notes: The Turkish asci are longer than described by Saccardo & Saccardo (1905: asci 55–75 × 10–12 µm), but the ascomata and ascospores are similar (Saccardo & Saccardo 1905: ascomata 70–150 µm diam.; ascospores 16–22 × 3–5 µm).

****Oletheriostrigula papulosa*** (Durieu & Mont.) Huhndorf & R.C. Harris, Brittonia 48: 551. 1996. Fig. 4

Ascomata amphigenous, numerous, immersed, black, subglobose, 130–150 µm diam. Asci 8-spored, paraphysate, short clavate, 65–85 × 10–12(–12.5)



Figs. 3–5. (3) ***Metasphaeria errabunda:*** ascus with ascospores. (4) ***Oletheriostrigula papulosa:*** a. asci; b. ascospore. (5) ***Neosetophoma samararum:*** conidia.

µm. Ascospores hyaline or pale, distichous, 3-septate, 4-guttulate, straight or slightly curved, ellipsoid-fusiform, 18–20 × 5–5.5 µm.

Material examined: **TURKEY**, **Bartin Province**, Peliken area, 66 m a. s. l., on living leaves of *Rhododendron ponticum* L., 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 14 (HBU).

Note: Asci in Bulgarian material of *O. papulosa* (Hüseyin et al. 2011: 65–85 × 13–15 µm) are wider than those in the Turkish material.

****Neosetophoma samararum*** (Desm.) Gruyter, Avescamp & Verkley, Mycologia 102: 1075. 2010. Fig. 5

Conidiomata immersed, erumpent, black, globose, 250–280 µm diam. Conidiophores hyaline, filiform, (18–)20–25 × 1 µm. Conidia biguttulate, unicellular, hyaline, cylindrical with rounded ends, 6–7.5 × 2–2.5 µm.

Material examined: **TURKEY**, **Bartin Province**, Peliken area, 70 m a.s.l., on the wing of fruit of *Acer campestre* L., 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 27 (HBU).

Notes: Conidia of *N. samararum* have been reported at 7 × 2.5 µm (Grove 1935; Gruyter et al. 2010), slightly wider than found in the Turkish material.

Figs. 6–8. (6) ***Cheiromyces stellatus:*** a. fragment of sporodochium; b. conidia. (7) ***Nectria viridescens:*** a. ascomata; b. asci with ascospores. (8) ***Physalospora eriostega:*** a. vertical section of ascomata; b. asci; c. ascospores.

****Cheiromyces stellatus*** Berk. & M.A. Curtis, Grevillea 3: 97. 1875. Fig. 6

Sporodochia scattered, single, superficial, flattened, circular or ellipsoidal, golden-brown, ≤210 µm diam. Conidiophores absent. Conidiogenous cells discrete, doliiform, pale brown, 4–5 × 3.5–4 µm. Conidia palmiform, consisting of truncated bottom basal cell, 4–4.5 µm wide, and (3–)5–12(–14) pale brown, vertical, slightly divergent or closely contiguous, more or less straight, cylindrical arms. Arms of the conidia 4–12(–14)-distoseptate with strongly reduced cell cavities, not constricted or only slightly constricted at septa, unbranched, straight, more or less of equal length, (22–)25–30(–56) × 4–5 µm. Apical cell of each arm almost hyaline and slightly longer and wider than the other cells of the arm.

Material examined: **TURKEY, Bartin Province**, Peliken area, 70 m a.s.l., on dead thin branches of *Ilex aquifolium*, 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 28 (HBU).

Notes: Arms of conidia in *C. stellatus* are given by Mel'nik (2000) as 23–40 × 4–7 µm, slightly wider than measured for the Turkish material.

Nectria viridescens C. Booth, Mycol. Pap. 73: 89. 1959. Fig. 7

Stromata ≤0.2 cm diam., densely, fleshy, reddish brown, erumpent from the bark. Perithecia bright-orange-red, with papillate ostiole, densely crowded, rounded, 170–210 µm diam. Asci 8-spored, surrounded by filiform paraphyses, cylindrical, 60–75 × 5–6 µm. Ascospores uniseriate, hyaline, uniseptate, not constricted, ellipsoidal, 6–7.5(–10) × 2.5–3 µm.

Material examined: **TURKEY, Bartin Province**, Peliken area, 66 m a.s.l., on dead branches of *C. betulus*, 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 3 (HBU).

Notes: The Turkish perithecial sizes fall within ranges previously reported for *N. viridescens:* 200–250 × 150–200 µm (Booth 1959), 125–200 µm diam

(Dennis 1981), and 270–350 × 250–350 µm (Byzova & Vasyagina 1981). Asci and ascospores in the Turkish material are smaller than reported elsewhere (Booth 1959: asci 68–76 × 6–9 µm; Dennis 1981: ascospores 7.5–10 × 4–5 µm).

Physalospora eriostega (Cooke & Ellis) Sacc., Syll. Fung. 1: 443. 1882. FIG. 8

Ascomata numerous, scattered, immersed, with only stomata erumpent, subepidermal, black, depressed-globose, 220–350 µm diam. Asci 8-spored, paraphysate, thick, short-stalked, broadly clavate, 80–100 × 15–20 µm. Ascospores distichous, hyaline, unicellular, broadly lanceolate, ellipsoidal, 25–33 × 9–12 µm.

MATERIAL EXAMINED: **TURKEY, BARTIN PROVINCE**, Peliken area, 80 m a.s.l., on dead branches of *Laurus nobilis* L., 16.IX.2012, A.S. Bülbül & E. Hüseyin EH-B 32 (HBU).

NOTES: The ascospores of *P. eriostega* on *L. nobilis* from Bartin province are smaller than those reported on *L. sassafras* L. by Saccardo (1882: 35 × 12 µm).

Discussion

The above eight species represent new records for Turkey and have not been included in previous publications on Turkish microfungi (Bremer & Petrak 1947; Bremer et al. 1948, 1952a,b; Petrak 1953, 1957; Karel 1958; Lohwag 1963; Göbelez 1963, 1964; Öner & Ekmekçi 1974; Tamer & Öner 1978; Baydar 1982; Öner et al. 1984; Tamer et al. 1989, 1990; Güven & Tamer 1993; Altan and Tamer 1996; Hüseyinov & Selçuk 1999; Hüseyin & Selçuk 2001; Hüseyin et al. 2003, 2009; Melnik et al. 2004; Erdoğdu & Hüseyin 2008; Stoykov & Denchev 2007; Göçmen et al. 2011). *Cheiromyces* Berk. & M.A. Curtis, *Neosetophoma* Gruyter et al., and *Oletheriostrigula* Huhndorf & R.C. Harris represent new generic records for Turkey.

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We thank Dr. Eugene Yurchenko (Pinsk, Belarus) and Dr. Sevda Kirbag (Elazig, Turkey) for serving as presubmission reviewers. Especially we are grateful to Dr. Shaun Pennycook (Auckland, New Zealand) for detailed linguistic help and nomenclatural review of the manuscript.

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Two new species of *Repetophragma* from the Iberian Peninsula

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Abstract — *Repetophragma fragile* and *R. ibericum,* two new microfungi collected on plant debris, are described and illustrated. *Repetophragma fragile* is distinguished by variable, subnavicular, obovoid to subrhomboidal, collapsing, 0–1-septate, pale brown conidia often with faint bands or irregular spots on the surface. *Repetophragma ibericum* is characterized by turbinate to globose, mostly 2-septate, guttulate conidia, with brown central cell and paler brown end cells.

Key words —asexual fungi, systematics, litter

Repetophragma Subram. was erected to accommodate *Sporidesmium* species with euseptate conidia produced on monoblastic, integrated, indeterminate conidiogenous cells with several percurrent extensions (Subramanian 1992). Castañeda-Ruiz et al. (2011) provided an illustrated update and key to *Repetophragma* species, adding another species and making twelve new combinations. During two field expeditions in Spain (to Burgos, 2010) and Portugal (to Minho province, 2011), more than 300 samples of dead plant material colonized by anamorphic fungi were collected. Plant debris was treated according to Castañeda-Ruiz (2005) and examined periodically under the stereomicroscope for a 2-month period. Semi-permanent and permanent microscope slides of fungi growing on the natural substrate were mounted in lactic acid 85% and polyvinyl alcohol and examined under the light microscope. Photomicrographs were obtained with a Zeiss AXIO Imager M1 light microscope (Göttingen, Germany). Among these 300 samples two

undescribed *Repetophragma* species were collected, which are herein described and illustrated.

Taxonomy

Repetophragma fragile R.F. Castañeda, Hern.-Rest., Gené & Guarro, **sp. nov.**

MycoBank MB802412 Figs 1, 2

Differs from *Repetophragma biseptatum* by having variable, fragile, very often collapsed, 0–1-septate, pale brown conidia, sometimes with faint bands or irregular spots on the surface.

Type: Spain, Burgos, Salas de los Infantes, Rio Pedroso, 42°04′N 3°18′W, on submerged decaying wood of unidentified plant, 10 November 2010, coll. M. Hernández-Restrepo & J. Gené C10/126 (**Holotype**, CBS H-20750; **Isotype**, FMR 11581).

Etymology: Latin, *fragile*, referred to the delicate, very often collapsed conidia.

Colonies on the natural substrate effuse, hairy, brown. Mycelium superficial and immersed, composed of septate, branched, brown, smooth hyphae 1–2.5 µm diam. Conidiophores distinct, single, erect, straight or slightly geniculate toward the apex, 6–12-septate, with 4–8 annellations near the apex, 87–190 × 5–10 µm, smooth, brown at the base and pale brown toward the apex. Conidiogenous cells monoblastic, terminal, integrated, 5–20 × 5–8 µm, indeterminate, annellidic with numerous cyathiform enteroblastic percurrent extensions. Conidial secession schizolytic. Conidia solitary, variable, subnavicular, obovoid, mucronate to somewhat acute, long subrhomboidal, fragile, very often collapsed, 0–1-septate, pale brown, but sometimes with faint bands or irregular spots, slightly obscure or brown pigmented on the surface, 25–42 × 10–17 µm, truncate, 5–7.5 µm wide at the base, smooth-walled, dry. Teleomorph unknown.

Repetophragma fragile superficially resembles *R. biseptatum* (M.B. Ellis) Subram. (Subramanian 1992, Castañeda-Ruiz et al. 2011), but *R. biseptatum* produces navicular, 3-septate, smooth-walled conidia with brown to dark brown upper cells and subhyaline basal cell, 17–23 × 7–8 µm and cannot be confused with *R. fragile*.

Repetophragma ibericum R.F. Castañeda, Hern.-Rest., Gené & Guarro, **sp. nov.**

MycoBank MB802415 Figs 3, 4

Differs from *Repetophragma quadriloculare* by its globose to subturbinate, mostly 2-septate conidia, with dark brown or brown central cell and pale brown end cells.

Type: Portugal, Minho province, "Lagoas de Bertiandos", 41°46′N 8°38′W, on bark of *Eucalyptus globulus* Labill, 9 November 2011, R.F. Castañeda, M. Hernández- Restrepo, J. Gené & J. Mariné-Gené C11/55 (**Holotype**, HAL 2452 F; **Isotype**, FMR 12183).

Etymology: Latin, *ibericum*, in reference to Iberian Peninsula.

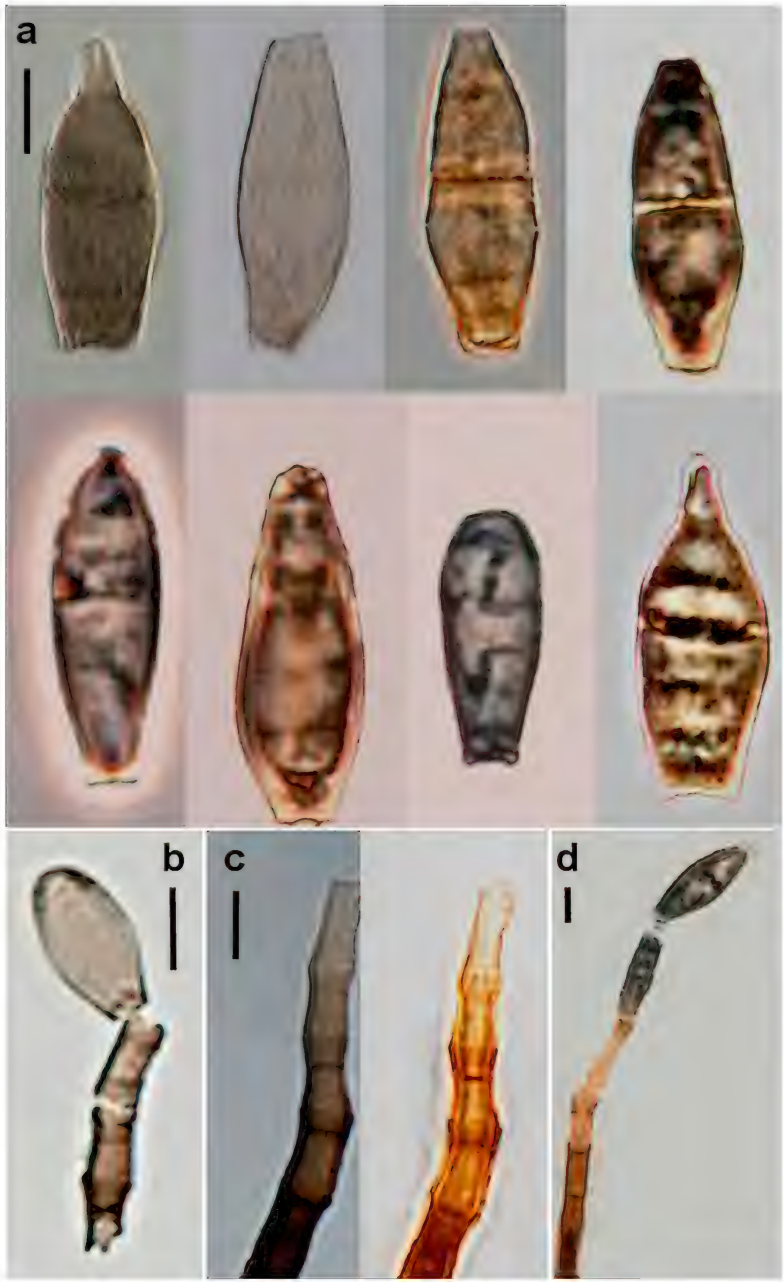

Fig. 1. *Repetophragma fragile* (ex holotype CBS H-20750):
a. Conidia. b, d. Conidiogenous cells and conidia. c. Conidiogenous cells. Bars = 10 µm.

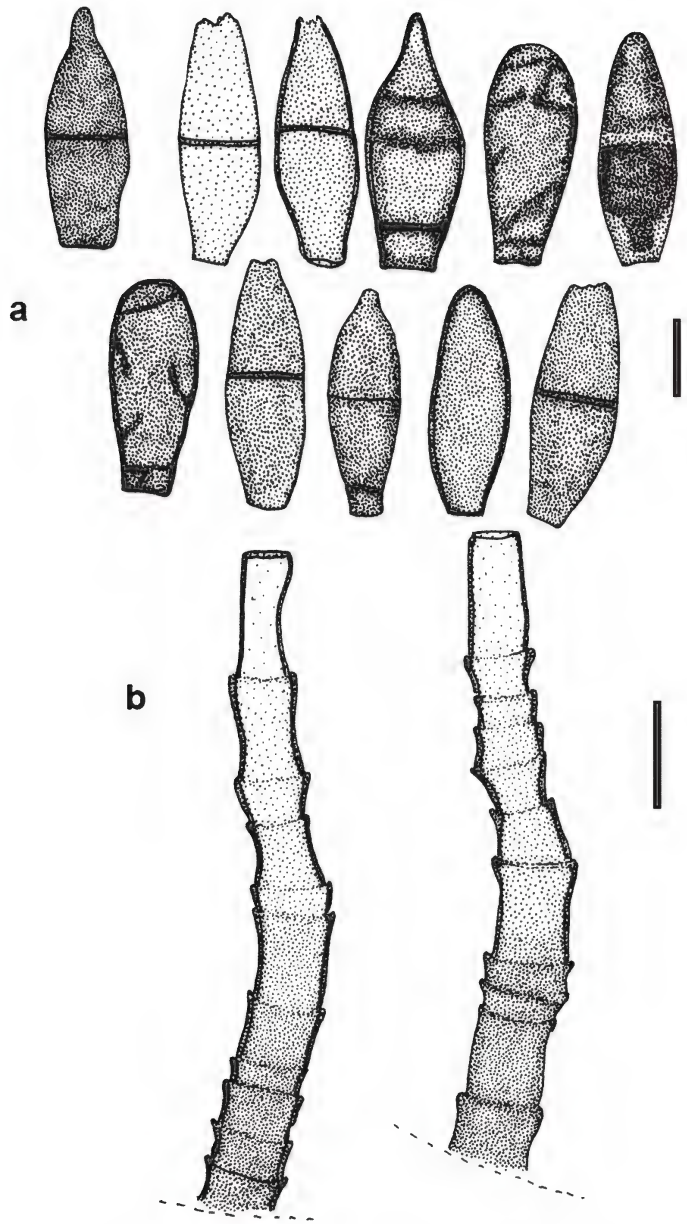

Fig. 2. *Repetophragma fragile* (ex holotype CBS H-20750):
a. Conidia. b. Conidiogenous cells. Bars = 10 µm.

Fig. 3. *Repetophragma ibericum* (ex holotype HAL 2452F):
a. Conidia. b. Conidiogenous cells. c–d. Conidiophores, conidiogenous cells, and conidia.
Bars = 10 µm.

Colonies on the natural substrate effuse, hairy, black. Mycelium superficial and immersed, composed of septate, branched, brown, smooth hyphae, 3–4 µm diam. Conidiophores distinct, single or sometimes fasciculate, unbranched, erect, straight or slightly geniculate toward the apex, 4–7-septate, with up to 7 annellations near the apex, 100–130 × 4.5–7 µm, dark brown at the base and pale brown above. Conidiogenous cells monoblastic, terminal, integrated, indeterminate, annellidic with numerous cyathiform to infundibuliform enteroblastic percurrent extensions. Conidial secession schizolytic. Conidia solitary, turbinate to globose, with broad obconical to somewhat cuneate, truncate basal cell, (1–)2(–3)-septate, dark brown at the septa, with brown to dark brown central cell and pale brown end cells, 14–18 × 6–8 µm, smooth-walled, dry. Teleomorph unknown.

Repetophragma ibericum is somewhat similar to *R. quadriloculare* (Matsush.) R.F. Castañeda et al. (Castañeda-Ruiz et al. 2011), but the latter has larger, obovoid to subglobose, 3-septate, smooth-walled conidia with dark brown central and basal cells, and a pale brown apical cell, 16–21 × 8–13 µm, from

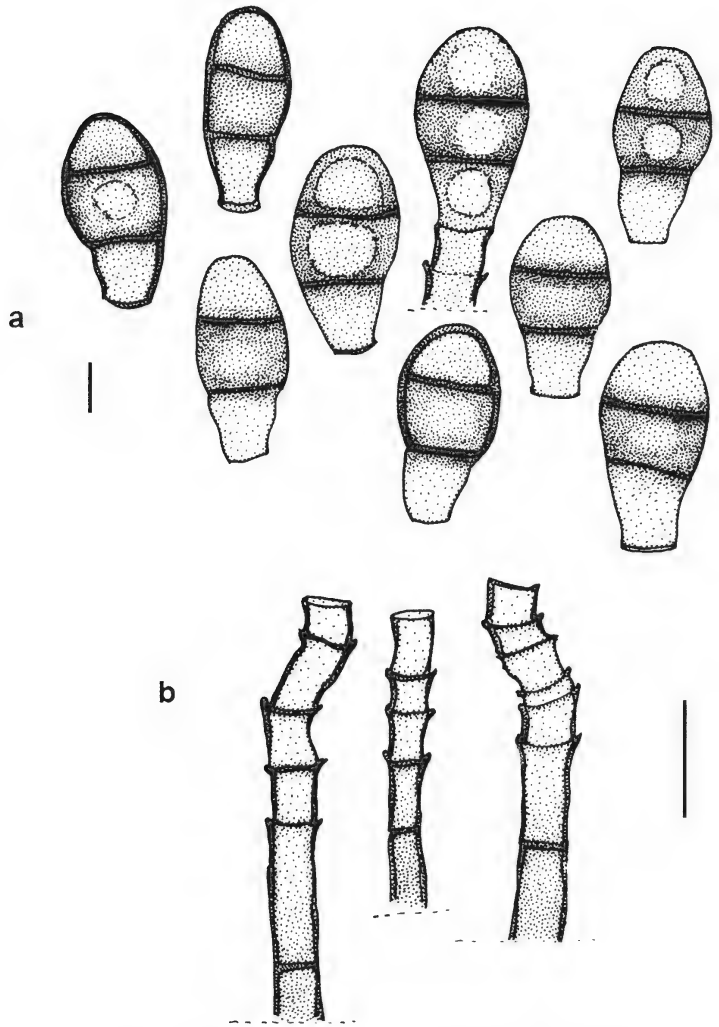

Fig. 4. *Repetophragma ibericum* (ex holotype HAL 2452F):
a. Conidia. b. Conidiogenous cells. Bars = 10 µm.

cultures on corn meal agar (conidia larger, 20–24 × 11–14 µm on the natural substrate, according to Matsushima 1993), so the two species can be easily separated.

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New records of *Rhizocarpon* from China

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Abstract – Four new lichen records are reported from China — *Rhizocarpon grande*, *R. infernulum*, *R. petraeum*, and *R. rubescens*. Detailed taxonomic descriptions with photos and comments are provided.

Key words – *Rhizocarpaceae*, Asia, taxonomy

Introduction

De Candolle originally established *Rhizocarpon* (*Rhizocarpaceae*) in 1805 (Kirk et al. 2008). The lichen genus, which is predominately distributed in temperate, alpine and polar regions, includes about 200 species worldwide (Feuerer & Timdal 2004; Ihlen 2004; Kirk et al. 2008). The genus is distinguished by its rock dwelling crustose thallus with black lecideine apothecia, branched and anastomosed paraphyses, *Rhizocarpon*-type asci, and 1-septate to muriform ascospores with a swollen perispore (halonate).

Traditionally *Rhizocarpon* is divided into taxa with a yellow thallus containing rhizocarpic acid (subgenus *Rhizocarpon*) and taxa with white, gray, or brown thalli lacking rhizocarpic acid (subgenus *Phaeothallus*) (Thomson 1967).

In China, 28 *Rhizocarpon* species have been reported (Wei 1991; Abbas & Wu 1998; Aptroot 2002; Aptroot & Sparrius 2003). During our study of *Rhizocarpon* in China, four additional species were found in subgenus *Phaeothallus*: *R. grande, R. infernulum, R. petraeum,* and *R. rubescens.*

Materials & methods

The examined specimens are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University). Their morphological and anatomical characters were examined under a stereomicroscope (COIC XTL7045B2) and a polarizing microscope (Olympus CX41). Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with solvent system C (Orange et al. 2001). Photographs were taken under Olympus SZX16 and BX61 with DP72.

Taxonomy

Rhizocarpon grande (Flörke ex Flot.) Arnold, Flora 54: 149 (1871) FIGS 1, 2

Thallus saxicolous, crustose, verrucose-areolate to bullate-verrucose, 0.15–0.2 mm thick, pale gray to gray-brown, epruinose; hypothallus prominent, black; cortex and medulla with obvious crystals; algal layer even. Apothecia lecideine, black, between the areolae, angular or rounded, 0.4–0.6 mm in diam; margin thin to disappearing; disc flat to convex; exciple brown, with crystals; epihymenium olivaceous brown, with crystals, usually K+ purple red; hymenium hyaline to pale brown in upper part, 110–135 μm tall; paraphysis

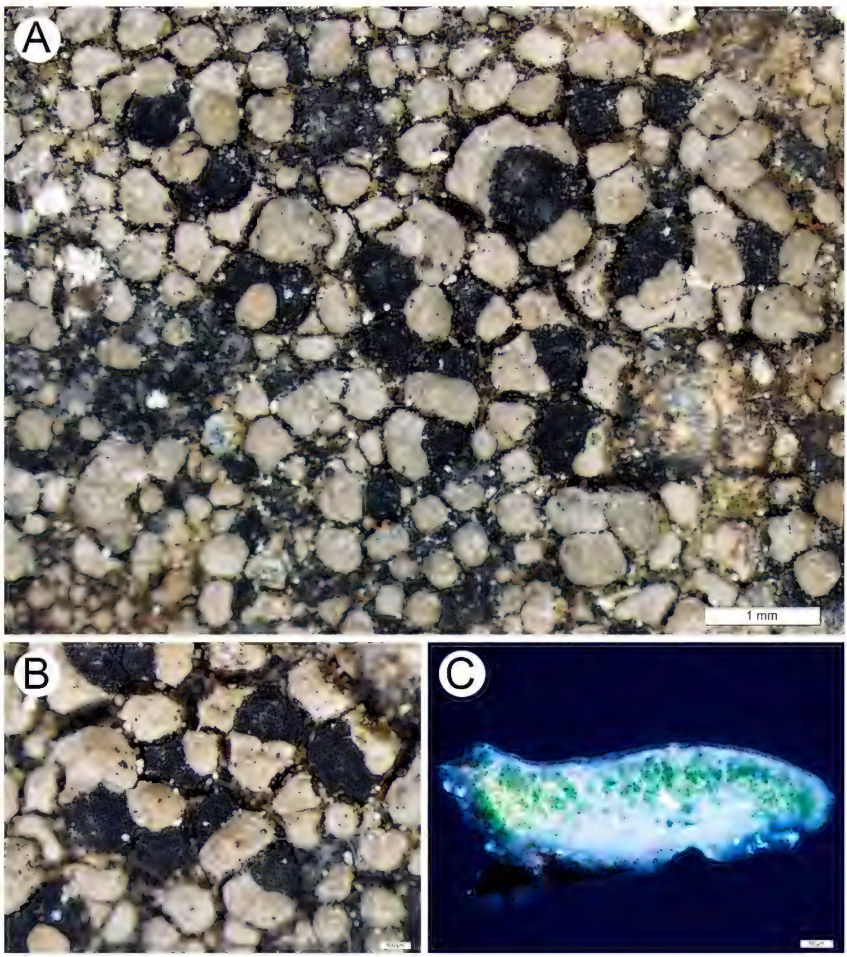

FIGURE. 1 *Rhizocarpon grande* (Fu 20124496, SDNU). A: Thallus; B: Apothecia; C: Crystals in thallus section. Scale bars: A = 1 mm; B = 500 μm; C = 50 μm.

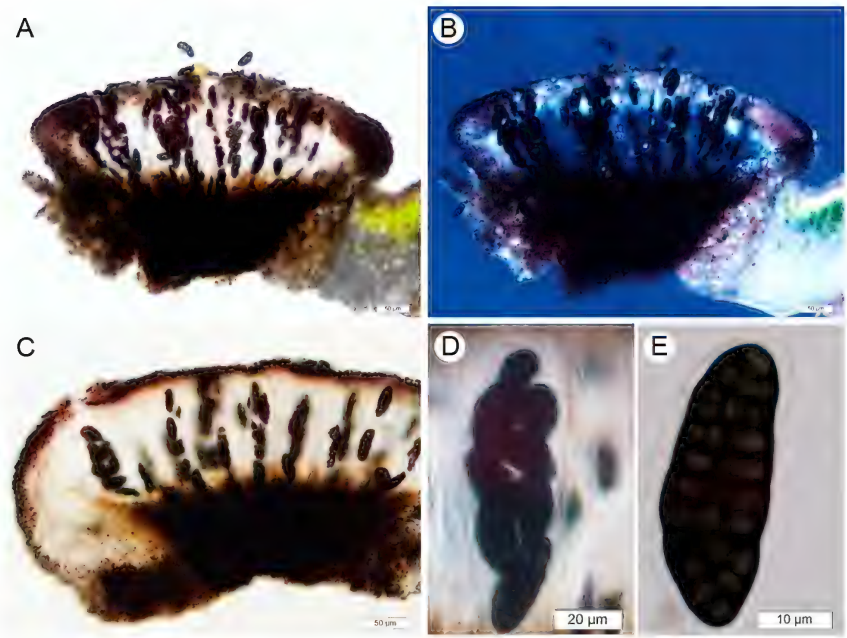

FIGURE. 2 *Rhizocarpon grande* (Fu 20124496, SDNU). A: Apothecium section; B: Crystals in apothecium section; C: K reaction; D: Ascus; E: Ascospore. Scale bars: A–C = 50 µm; D = 20 µm; E = 10 µm.

capitate, branched and anastomosing; hypothecium brown. Asci *Rhizocarpon*-type, 8-spored. Ascospores dark brown, muriform, ellipsoid, 25–40 × 10–16 µm.

CHEMISTRY — Secondary metabolites: gyrophoric acid and stictic acid.

SPECIMEN EXAMINED — **CHINA. INNER MONGOLIA**, Arxan city, Mt. Jiguan, alt. 1500 m, on rock, 29 Aug. 2011, H.D. Fu 20124496 (SDNU).

COMMENTS — *Rhizocarpon grande* resembles *R. eupetraeum* in having a verrucose thallus, but *R. eupetraeum* contains norstictic acid, lacks gyrophoric acid, and has a K– epihymenium. *Rhizocarpon grande* is also similar to *R. geminatum*, which can be differentiated by its 2-spored asci, larger ascospores (35–60 × 18–30 µm; Thomson 1997), and absence of gyrophoric acid.

DISTRIBUTION — *Rhizocarpon grande* has been reported from North America, Europe, Asia, and Antarctica (Thomson 1997, Galloway 2007, Golubkov & Matwiejuk 2009). New to China.

Rhizocarpon infernulum (Nyl.) Lynge, Rhodora 36:158 (1934) FIGS 3, 4

Thallus saxicolous, crustose, rimose to cracked areolate, brown, 0.05–0.085 mm thick, epruinose; prothallus black, well developed; cortex without crystals,

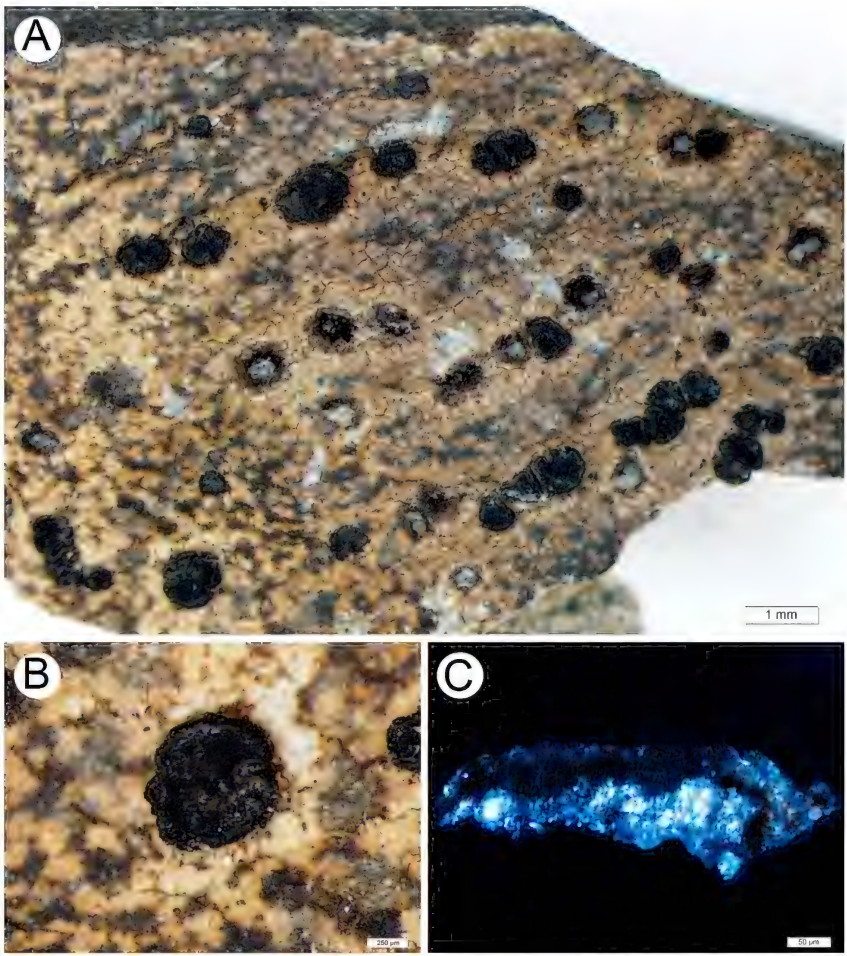

FIGURE. 3 *Rhizocarpon infernulum* (Zhang 20103078, SDNU). A: Thallus; B: Apothecium; C: Crystals in thallus section. Scale bars: A = 1 mm; B = 250 μm; C = 50 μm.

medulla with obvious crystals; algal layer even. Apothecia lecideine, black, innate, 0.3–0.7 mm in diam; disc flat; margin persistent, thin; exciple well developed, carbonaceous, without crystals; epihymenium usually with aeruginous blue to blue-black pigment, without crystals, N+ purple red; hymenium hyaline, 70–90 μm tall; paraphysis branched and anastomosing, scarcely swelling at apex, with sharply delimited, brown cap, although this is often masked by a blue-green epithecial pigment; hypothecium brown. Asci *Rhizocarpon*-type, 8-spored. Ascospores hyaline, 1-septate, ellipsoid, 15–18 × 7–8.5 μm.

CHEMISTRY —No substances were detected by TLC.

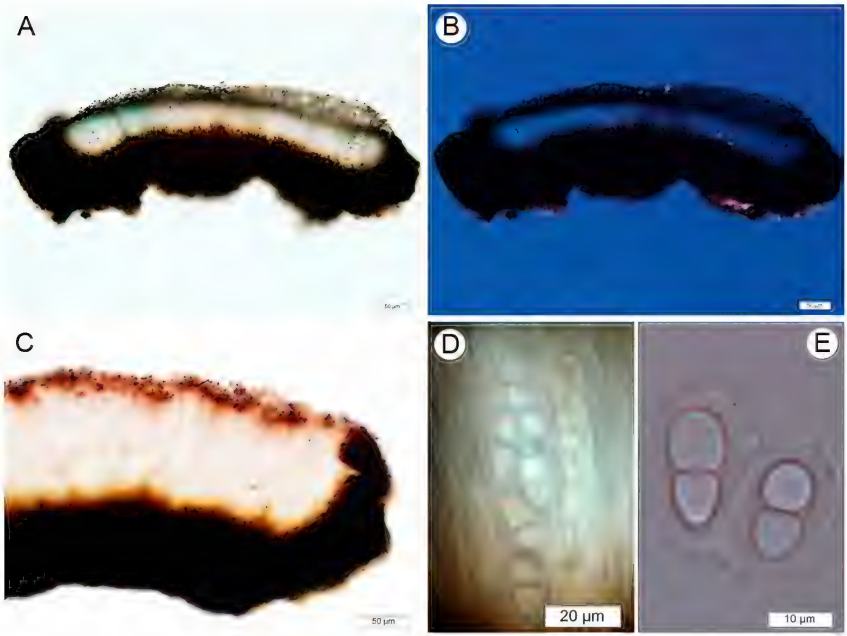

FIGURE. 4 *Rhizocarpon infernulum* (Zhang 20103078, SDNU). A: Apothecium section; B: Crystals in apothecium section; C: N reaction; D: Ascus; E: Ascospores. Scale bars: A–C = 50 µm; D = 20 µm; E = 10 µm.

SPECIMENS EXAMINED — **CHINA. GUIZHOU**, Leishan county, Mt. Leigong, alt. 2700 m, on rock, 9 Nov. 2009, L.L. Zhang 20103078 (SDNU); alt. 2100 m, on rock, 1 Apr. 2011, X.R. Kou 20111813 (SDNU); alt. 1800 m, on rock, 2 Apr. 2011, Y.L. Cheng 20112591A (SDNU).

COMMENTS — *Rhizocarpon infernulum* is morphologically close to *R. hochstetteri*, which can be separated by the thicker thallus (0.1–0.35 mm thick) and larger ascospores (21–25 × 10–12 µm) (Fryday 2002). *Rhizocarpon infernulum* is also similar to *R. cinereovirens*, which can be distinguished by its less well-developed exciple with a hyaline interior and the usual presence of norstictic acid or stictic acid.

DISTRIBUTION — *Rhizocarpon infernulum* has been reported from Europe, Asia, and North America (Fryday 2002, Fletcher et al. 2009). New to China.

Rhizocarpon petraeum (Wulfen) A. Massal., Ric. Auton. Lich. Crost. 102 (1852) FIGS 5, 6

Thallus saxicolous (usually on siliceous rock), crustose, continuous to rimose, areolate in part, often flat and polygonal, 0.14–0.2 mm thick, pruinose, when eroded, thallus surface gray to brown; when eroded, thallus surface gray

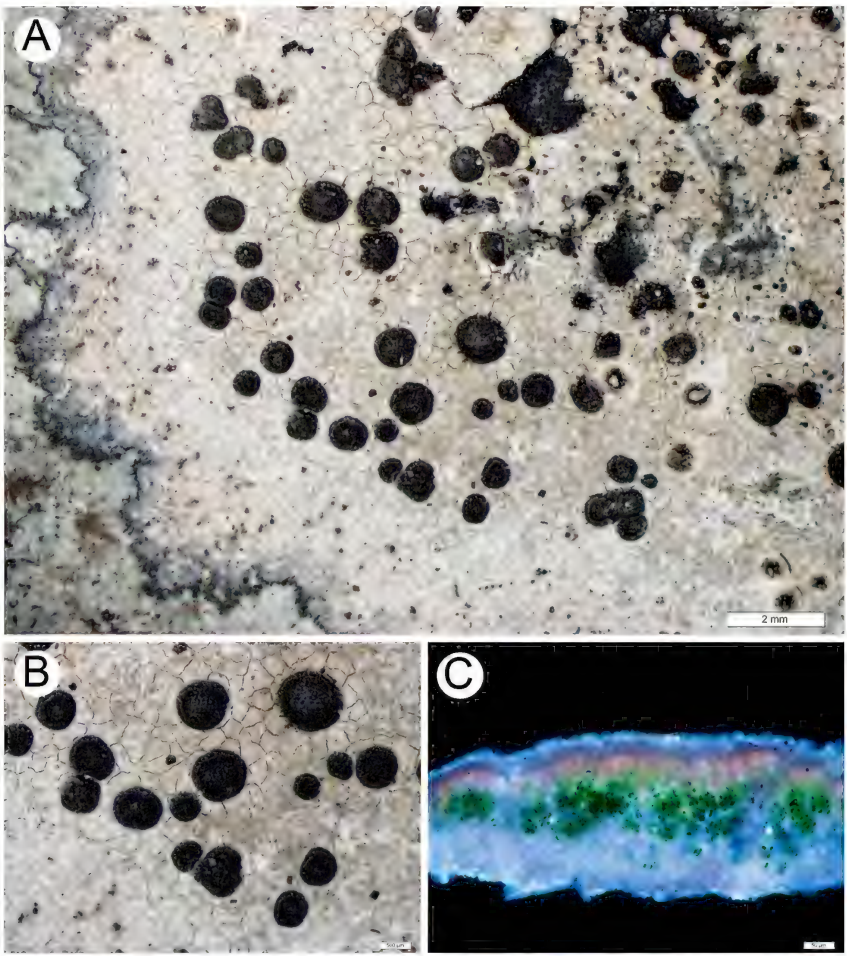

FIGURE. 5 *Rhizocarpon petraeum* (Li 20126494A, SDNU). A: Thallus; B: Apothecia; C: Crystals in thallus section. Scale bars: A = 2 mm; B = 500 μm; C = 50 μm.

to brown; prothallus distinct, black; cortex and medulla with obvious crystals; algal layer even. Apothecia lecideine, innate, irregularly arranged, 0.4–1 mm diam; disc flat, black, epruinose; margin distinct, persistent; exciple dark brown to dark blue-green at the rim, inner part colourless to brown, with crystals, N+ purple; epihymenium olivaceous, with obvious crystals; hymenium hyaline, 150–200 μm tall; paraphysis branched and anastomosing; hypothecium brown. Asci *Rhizocarpon*-type, 8-spored. Ascospores: hyaline, becoming dark when over-mature, narrowly ellipsoid to ellipsoid, eumuriform, 23–50 × 12–16 μm, with 12–21 cells in optical view.

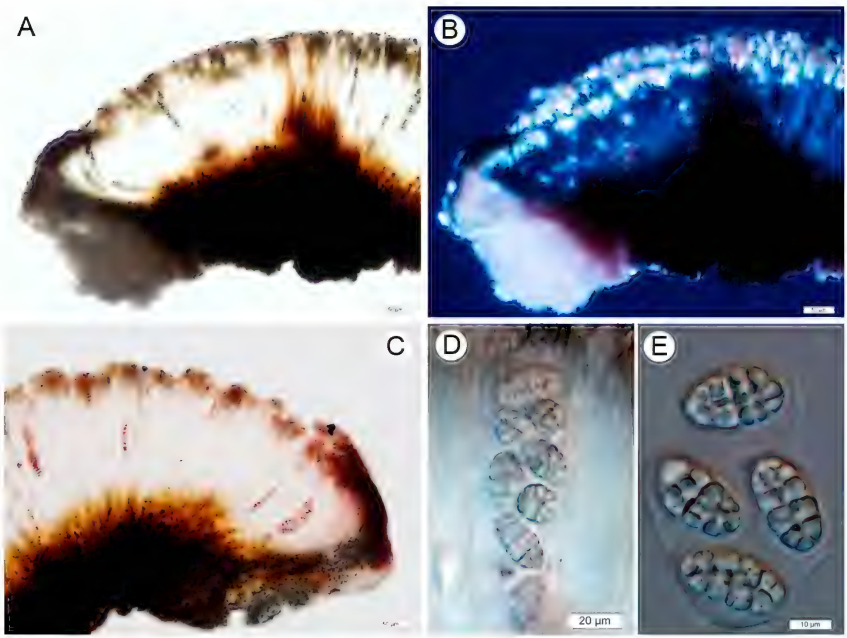

FIGURE. 6 *Rhizocarpon petraeum* (Li 20126494A, SDNU). A: Apothecium section; B: Crystals in apothecium section; C: N reaction; D: Ascus; E: Ascospores. Scale bars: A–C = 50 µm; D = 20 µm; E = 10 µm.

CHEMISTRY — Secondary metabolites: stictic acid.

SPECIMENS EXAMINED — **CHINA. XINJIANG**, Urumqi, Nanshan–Xiaoquzi, alt. 2700 m, on rock, 28 Aug. 2011, L. Li 20126494A (SDNU); 28 Aug. 2011, Z.L. Huang 20125928 (SDNU).

COMMENTS — *Rhizocarpon petraeum* is morphologically close to *R. umbilicatum*, which can be distinguished by its smaller ascospores (22–27.5 × 11–15 µm) with 6–8 cells in optical view and usual growth on calcareous rock (Ihlen 2004). When the thallus is not distinctly covered with pruina, *R. petraeum* is also similar to *R. lavatum* and *R. reductum*, but *R. lavatum* lacks secondary metabolites, and *R. reductum* produces ascospores containing fewer cells (8–13 cells in optical view; Ihlen 2004).

DISTRIBUTION — *Rhizocarpon petraeum* has been reported from Europe, North America, Australia, and Asia (Ihlen 2004, Fletcher et al. 2009, Golubkov & Matwiejuk 2009). New to China.

Rhizocarpon rubescens Th. Fr., Lich. Scand. 1: 631 (1874) FIGS 7, 8

Thallus saxicolous, crustose, areolate, areoles convex, rounded, containing small granules, gray to gray-brown, 0.15–0.2 mm thick, epruinose; prothallus

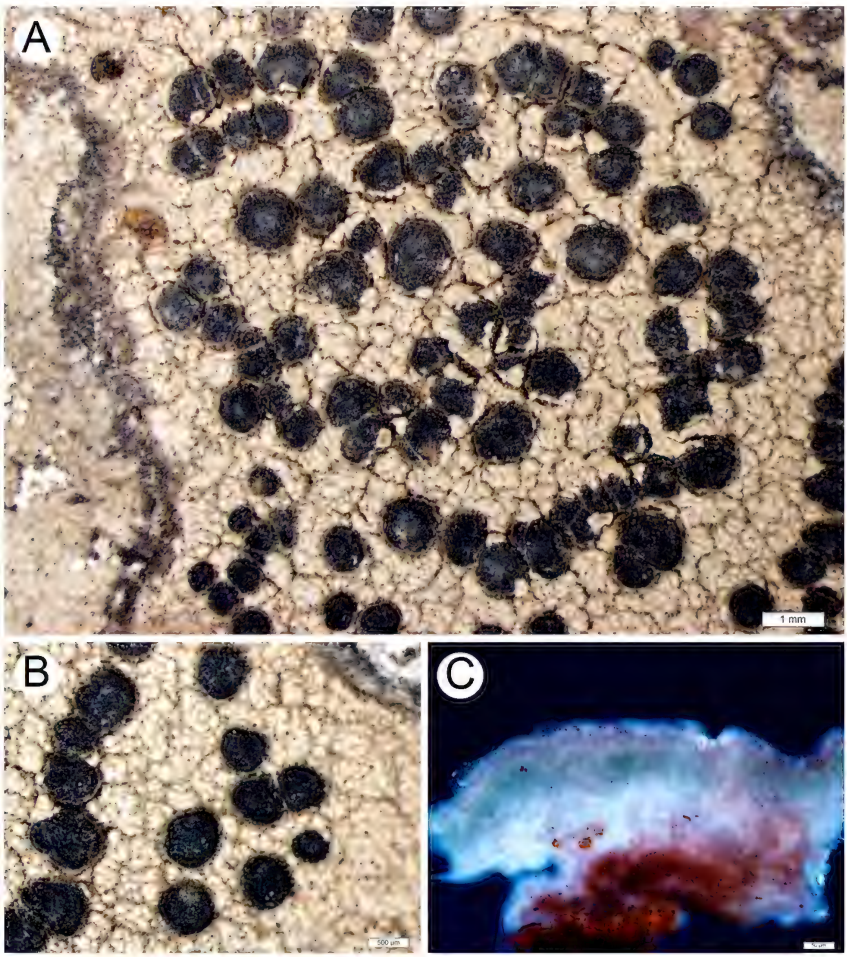

FIGURE. 7 *Rhizocarpon rubescens* (Shi 061473, SDNU). A: Thallus; B: Apothecium; C: Crystals in thallus section. Scale bars: A = 1 mm; B = 500 µm; C = 50 µm.

distinct, black; cortex and medulla with crystals; algal layer even. Apothecia lecideine, innate, irregularly arranged or sometimes arranged in a circular pattern, 0.5–1mm diam; disc flat, black; margin distinct when young; exciple dark brown, with obvious crystals, K+ purple; epihymenium dark green, with crystals; hymenium hyaline, often with a Macrocarpa-green tinge, 130–150 µm tall; paraphysis branched and anastomosing; hypothecium dark brown. Asci *Rhizocarpon*-type, 8-spored. Ascospores hyaline, ellipsoid, eumuriform, 25–38 × 12–15 µm.

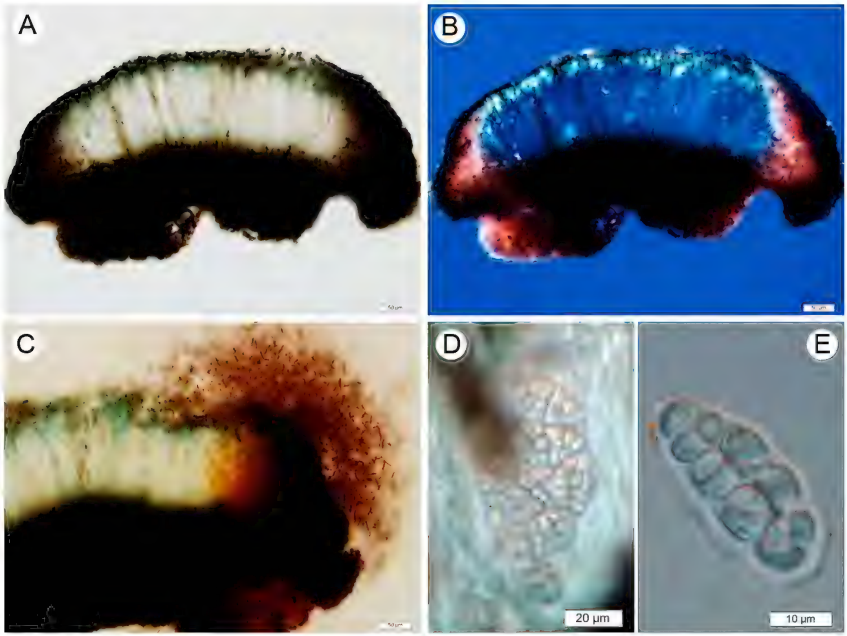

FIGURE. 8 *Rhizocarpon rubescens* (Shi 061473, SDNU). A: Apothecium section; B: Crystals in apothecium section; C: K reaction; D: Ascus; E: Ascospore. Scale bars: A–C = 50 µm; D = 20 µm; E = 10 µm.

CHEMISTRY — Secondary metabolites: norstictic acid.

SPECIMENS EXAMINED — **CHINA. GANSU**, Wenxian county, Qiujiaba, alt. 2250 m, on rock, 3 Aug. 2006, X.L. Shi 061473 (SDNU), 061475 (SDNU); **YUNNAN**, Lijiang city, Mt. Laojun, alt. 3800 m, on rock, 5 Nov. 2009, H.Y. Wang 20100308A (SDNU).

COMMENTS — *Rhizocarpon rubescens* resembles *R. suomiense* in having hyaline, muriform ascospores and norstictic acid, but *R. suomiense* has 2-spored asci and strongly muriform ascospores (31–54 cells in optical view; Ihlen 2004). *Rhizocarpon rubescens* is morphologically close to *R. reductum*, but *R. reductum* contains stictic acid and has a K+ blue epithecium and K+ yellow exciple.

DISTRIBUTION — *Rhizocarpon rubescens* has been reported from Europe and North America (Fryday 2000, Ihlen 2004). New to China.

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Digitella rigidophora and *Redbia inflata*, two new microfungi from Mexico

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Abstract — Two microfungi collected from plant debris in cloud forests during a survey of the fungi associated with leaf litter in Veracruz are described and illustrated. *Digitella rigidophora* gen. et sp. nov. is distinguished by polytretic conidiogenous cells having a successive sympodial but rectilinear or geniculated proliferation and with discoid to papillate-perforate thickened conidiogenous loci and digitate solitary dark brown to brown conidia. *Redbia inflata* sp. nov. is characterized by alternate, curved to coiled, slightly echinulate branches that possess a distinct ampulla at the ends and by coarsely verruculose, subhyaline, 0–1-septate conidia.

Key words —anamorphic fungi, taxonomy, tropical fungi

Introduction

Tropical mountain cloud forests are among the most diverse biomes in the world. In Mexico, although this kind of forest covers less than 1% of the land surface, it contains 10–12% of the flowering plant species in the country (Rzedowsky 1996). Because of the climatological conditions, all kinds of fungi grow in a wide variety of habitats provided by these forests. Unfortunately, such forests are severely threatened in Mexico. Given that very few mycological studies focused on microfungi have been made in this diverse ecosystem, it is urgent to explore the few areas where there are still remnants of forest. Continuing with a long-term study of anamorphic fungi from tropical mountain cloud forests of central Veracruz State, we sampled fungi in several forest fragments and found two interesting fungi colonizing the rachis of a palm tree and leaves of an unidentified member of the *Lauraceae*. They are described and discussed here.

Material & methods

During a foray to study tropical forest mycobiota, 110 samples of plant litter were collected near the San Martin Tuxtla Volcano (1070 m alt., 18°32′06″N 95°08′25.46.2″W) and Santa Marta, Soteapan (1375 m alt., 18°20′54″N 94°53′23″W). Individual plant samples were placed in paper or plastic bags taken to the laboratory and treated according to the techniques described by Castañeda Ruiz (2005). Mounts were prepared in polyvinyl alcohol-glycerol (8 g in 100 ml of water, plus 5 ml of glycerol) and measurements made at a magnification of ×1000. Photographs were obtained using a Nikon microscope with differential interference contrast (Eclipse E600) and a JEOL (JSM-S600LV) scanning electron microscope (SEM) using the techniques described by Figueras & Guarro (1988).

Taxonomy

Digitella R.F. Castañeda, Heredia & R.M. Arias, **gen. nov.**

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Differs from *Dendryphion* by successive enteroblastic percurrent proliferations after each holoblastic sympodial proliferation of the conidiogenous cells, by discoid to papillate-perforated strongly melanized conidiogenous loci, and solitary digitate brown to dark brown conidia, and differs from *Ceratosporella* by tretic conidiogenous cells.

Type species: *Digitella rigidophora* R.F. Castañeda et al.

Etymology: Latin, *Digitella*, referring to the finger-like conidial branches.

Anamorphic fungi. Colonies on the natural substratum effuse, hairy, dark brown or black. Mycelium partly superficial and immersed. Conidiophores distinct, single, unbranched, septate, dark brown or black, smooth or verrucose. Conidiogenous cells polytretic, integrated, terminal at first with a single terminal conidiogenous locus, then indeterminate, with successive sympodial rectilinear proliferations followed by enteroblastic percurrent proliferation, being the same process repeated several times, producing terminal and intercalary conidiogenous loci. Conidiogenous loci evident, convex, discoid, perforated, thick and black. Conidial secession schizolytic. Conidia solitary, acropleurogenous, digitate, euseptate, brown to dark brown, each with a subconical to somewhat campanulate, conspicuously cicatrized, strongly melanized, subtruncate basal cell.

Digitella rigidophora R.F. Castañeda, Heredia & R.M. Arias, **sp. nov.**

MycoBank 800395 Fig. 1A–G

Differs from *Dendryphion* spp. by successive enteroblastic percurrent proliferations after each holoblastic sympodial proliferation of the conidiogenous cells and solitary digitate brown to dark brown smooth conidia; differs from *Ceratosporella* spp. by tretic conidiogenous cells.

Type: Mexico, Veracruz, San Martin Tuxtla Volcano, on rachis of unidentified palm tree, 5 January 2011, G. Heredia (**Holotype**: XAL CB1692).

Etymology: Latin, *rigidophora*, referring to straight, rigid conidiophores.

Fig. 1. ***Digitella rigidophora*** (ex holotype XAL CB1692). a. Conidia. b. Conidiogenous cells and conidia. c. Conidiogenous locus with a pore (shown by arrow). d. Conidiogenous cells with several enteroblastic percurrent proliferations (shown by arrows). e. Conidiogenous cells and conidiogenous loci (SEM). f. Conidiophore (SEM). g. Conidia (SEM). ***Dendryphion digitatum*** (ex XAL CB1236). h. Conidiogenous cells and conidia. Bars = 10 μm, except where otherwise indicated.

Anamorphic fungus. Colonies on the natural substratum effuse, hairy, dark brown or black. Mycelium partly superficial and immersed. Conidiophores macronematous, mononematous, single, septate, 120–245 × 8–14 μm,

dark brown or black at the base, brown to pale brown at the apex, smooth. Conidiogenous cells polytretic, integrated, 8–11 × 8–12 µm, brown or pale brown, terminal at first with a single terminal conidiogenous locus, then indeterminate, with successive sympodial rectilinear proliferation, sometimes delimited by one septum and followed by enteroblastic percurrent proliferation, being the same process repeated several times, producing terminal and intercalary conidiogenous loci. Conidiogenous loci evident, convex, discoid, papillate-perforated, 5–7 µm diam., thick and black. Conidial secession schizolytic. Conidia solitary, acropleurogenous, digitate, euseptate, constricted at septate when mature, brown to dark brown, 49–78 × 23–26 µm, composed of two branches, 43–75 µm long and a subconical to somewhat campanulate, conspicuously cicatrized, strongly melanized, subtruncate basal cell, 8–10 µm wide. Teleomorph unknown.

Comments — The pattern of ontogeny in *Digitella* can be classified using the scheme for conidial development in Kirk et al. (2008). Conidial initiation is tretic, delimitation by 1 septum, secession schizolytic, maturation by diffuse wall-building, percurrent enteroblastic conidiogenous cell extension, followed by further conidial initiation by replacement of apical wall-building; strongly melanized, each successive conidium seceding before the next percurrent elongation of the conidiogenous cell, but sometimes also holoblastic, sympodial proliferation occurring, with two or more conidia produced. The combination of sympodial and enteroblastic percurrent proliferations with discoid, convex, thick, darkened conidiogenous loci in *Digitella* separates it from *Dendryphion* Wallr. (Bhat & Sutton 1985, Ellis 1971, 1976, Hughes 1978, Siboe et al. 1999), where dark cicatrized loci are produced after sympodial proliferations of the discrete, polytretic conidiogenous cells disposed in geniculate branches of the conidiophores (e.g., *Dendryphion digitatum* Subram., Fig. 1h). *Dendryphion* conidia are solitary or very often short catenulate and only digitate or cheiroid in few species. *Ceratosporella* Höhn. species also have digitate to cheiroid conidia similar to *Digitella*, but the conidium ontogeny in *Ceratosporella* is holoblastic and only enteroblastic percurrent proliferations occur during the conidiogenous events.

Redbia inflata Heredia, R.M. Arias & R.F. Castañeda, **sp. nov.** Fig. 2

MycoBank MB800396

Differs from *Redbia elegans* by curved to sub-coiled branches; swollen to botryose conidiogenous cells and 0–1-septate, oblong to cylindrical conidia, which sometimes bear secondary conidia at the apex.

Type: Mexico, Veracruz, Soteapan, Reserva de la Biosfera de la Sierra Santa Marta, on decaying leaf of an unidentified species of the *Lauraceae*, 5 August 2011, G. Heredia (**Holotype**: XAL CB1691).

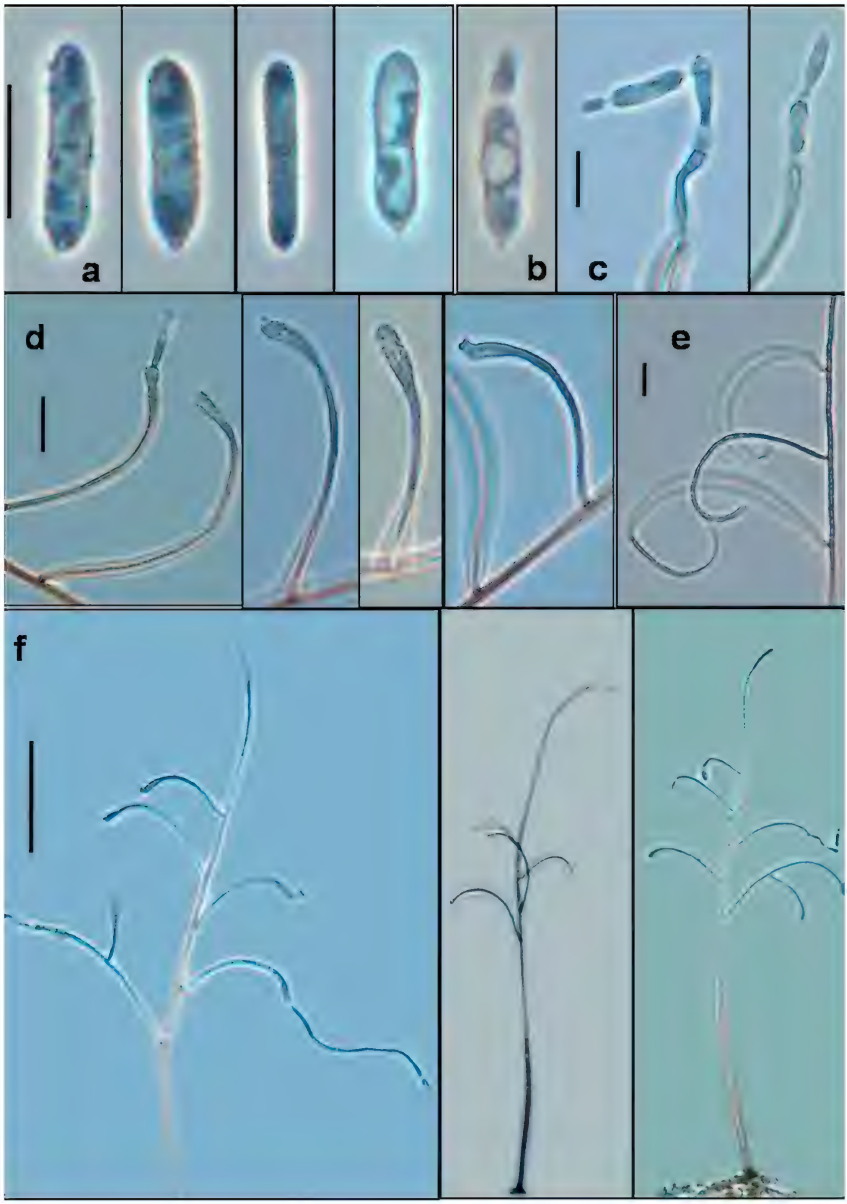

Fig. 2. ***Redbia inflata*** (ex holotype XAL CB1691). a. Conidia. b. Catenulate conidia. c. Conidiogenous cells and conidia. d, e. Branches. f. Conidiophores. Bars a, c = 10 µm; d, e = 40 µm; f = 100 µm.

Etymology: Latin, *inflata*, referred to the vesicular swellings at the tips of branches.

Colonies on the natural substratum, effuse, hairy, brown. Mycelium superficial and immersed. Hyphae septate, branched, 1–2 µm diam., smooth, pale brown to brown. Conidiophores macronematous, mononematous, erect, straight, cylindrical 14–19-septate, smooth at the base, verruculose to verrucose towards the apex and branch tips, 480–600 × 12–17 µm, alternately branched, with branches whip-like, curved, slightly coiled, brown at the base and subhyaline 94–200 µm long, echinulate, with an ampulla or a botryose to inflated apex 5–7 µm wide. Conidiogenous cells polyblastic, denticulate, terminal, integrated and discrete, subhyaline, 6–20 × 5–8 µm, indeterminate, with several sympodial proliferations bearing conspicuous denticles. Conidial secession schizolytic. Conidia solitary or sometimes short catenulate, oblong to somewhat cylindrical, coarsely verruculose to verrucose, obtuse or rounded at the apex, truncate at the base, 0–1-septate, 12–16 × 2.5–3.5 µm, subhyaline, dry. Teleomorph unknown.

Comments — Deighton & Pirozynski (1972) established *Redbia* based on a mycophilous fungus, *R. pucciniicola* (the type species), found on telia of *Puccinia holosericea* Cooke. *Redbia* is distinguished by conidiophore branches, brown below and paler towards the apex and branches, with sympodial proliferating, denticulate conidiogenous cells. Three additional species were described: *R. elegans* Piroz. & Hodges (Pirozynski & Hodges 1973), *R. laxa* R.F. Castañeda (Castañeda 1985), and *R. trichomambusta* R.W. Barreto (Barreto1994). *Redbia inflata* is superficially similar to *R. elegans* in conidial ornamentation, but *R. elegans* is easily differentiated by its narrow ellipsoid to fusiform, 3-septate, 15–35 × 4–5 µm conidia.

Key to Redbia species

1 Conidiophores simple, conidia obovate, 0–3-septate, 8–21 × 1–6 µm, smooth, pale brown ..... *R. trichomambusta*
Conidiophores branched 2

2 (1) Conidia smooth 3
Conidia verruculose 4

3 (2) Conidia fusiform, 1–3-septate, hyaline, 10–21 × 3–4.5 µm ..... *R. pucciniicola*
Conidia obovoid to clavate, 1-septate, sometimes unequal, hyaline or subhyaline, 7.5–10.5 × 3–3.5 µm ..... *R. laxa*

4(2) Conidia narrowly ellipsoid or fusiform, pale straw-colour, 3-septate, 15–35 × 4–5 µm ..... *R. elegans*
Conidia oblong to somewhat cylindrical, subhyaline, 0–1-septate, 12–16 × 2.5–3.5 µm ..... *R. inflata*

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A new coprophilous *Gilmaniella* species from Malaysia

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Abstract — *Gilmaniella nyukfahii* sp. nov., collected from cow dung in Malaysia, is described and illustrated. It differs from other *Gilmaniella* species in having globose conidia with 5–7 distinct germ pores.

Key words — dung fungi, dematiaceous hyphomycete, mitosporic fungi, saprotroph, taxonomy

Introduction

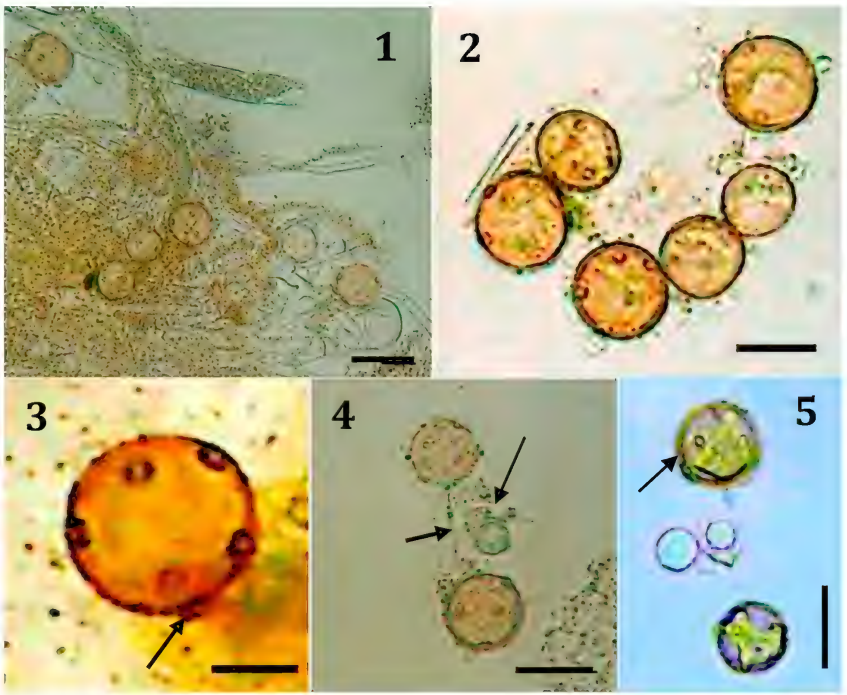
Species of *Gilmaniella* G.L. Barron (Barron 1964) are fungi that produce masses of dematiaceous conidia from superficial semi-macronematous and mononematous conidiophores (Ellis 1971). The conidia are unicellular, with one or several distinct germ pores (Umali et al. 1998). There are eight reported species of *Gilmaniella* (Index Fungorum 2013). Umali et al. (1998) reviewed and keyed the seven *Gilmaniella* species known at that date. Dubey et al. (2011) described a foliicolous species, *G. indica* Dubey et al., in which the conidia have a single germ pore similar to those of *G. humicola* G.L. Barron.

During a survey of fungal diversity from cow dung in Malaysia, we found an undescribed species of *Gilmaniella* in cow dung samples incubated at 28°C. This species is described here and compared with other *Gilmaniella* spp.; its holotype is conserved at the herbarium of the Centre for Biodiversity Research, Faculty of Science, Universiti Tunku Abdul Rahman (Perak campus), Kampar, Malaysia (UTAR).

Taxonomy

Gilmaniella nyukfahii Goh, L.L. Lee & K.C. Teo, **sp. nov.** Figs 1–11

MycoBank MB804095



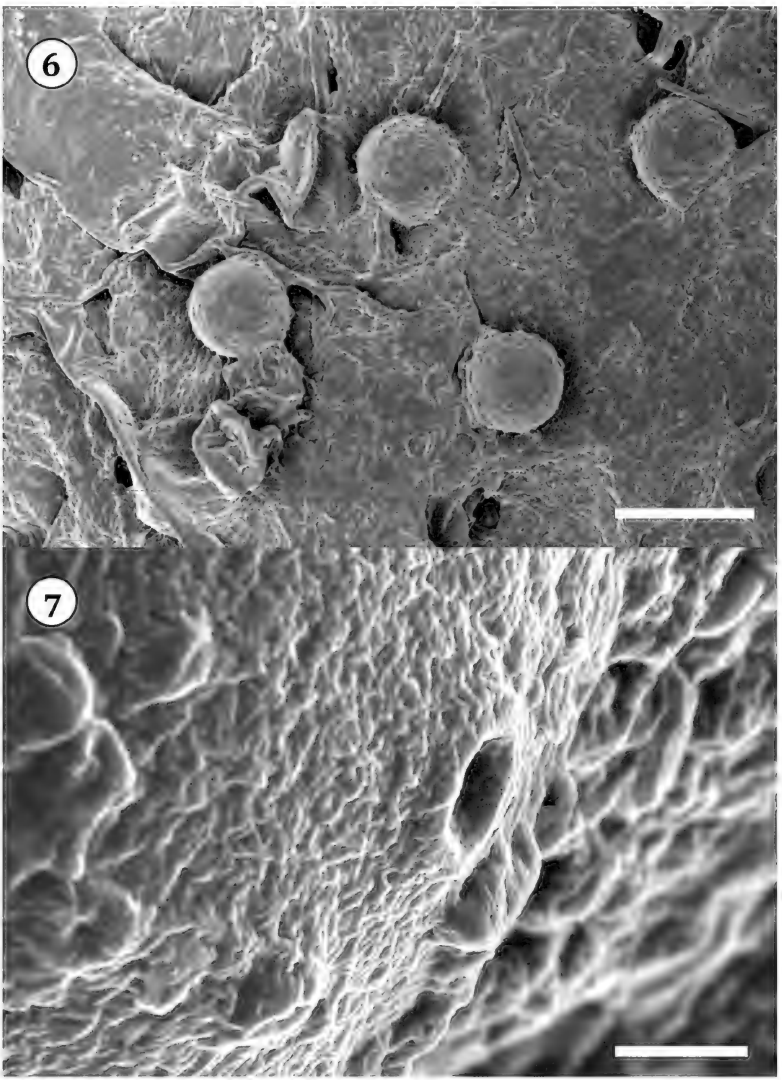
Figs 1–5. *Gilmaniella nyukfahii* (holotype). 1. Conidia from natural substratum, scattered among hyphae of other fungi (e.g., zygomycetes) and bacterial mass. 2. Conidia, each with multiple germ pores. 3. Conidium with six visible germ pores; arrow points to a protruding germ pore; the conidial wall is somewhat roughened. 4. Conidia with germ pores; the conidiophore (arrowed) is delicate, hyaline, and hypha-like. 5. Developing and near-mature conidia (showing germ pores); the conidia are thick-walled (arrowed). Scale bars: 1 = 20 µm; 2, 5 = 10 µm; 3 = 5 µm; 4 = 15 µm.

Differs from other species of *Gilmaniella* in having conidia with 5–7 distinct germ pores.

Type: Malaysia. Perak, Kampar, on cow dung incubated at 28°C, November 2012, Li-Ling Lee, (**Holotype**, UTAR(M)-0001).

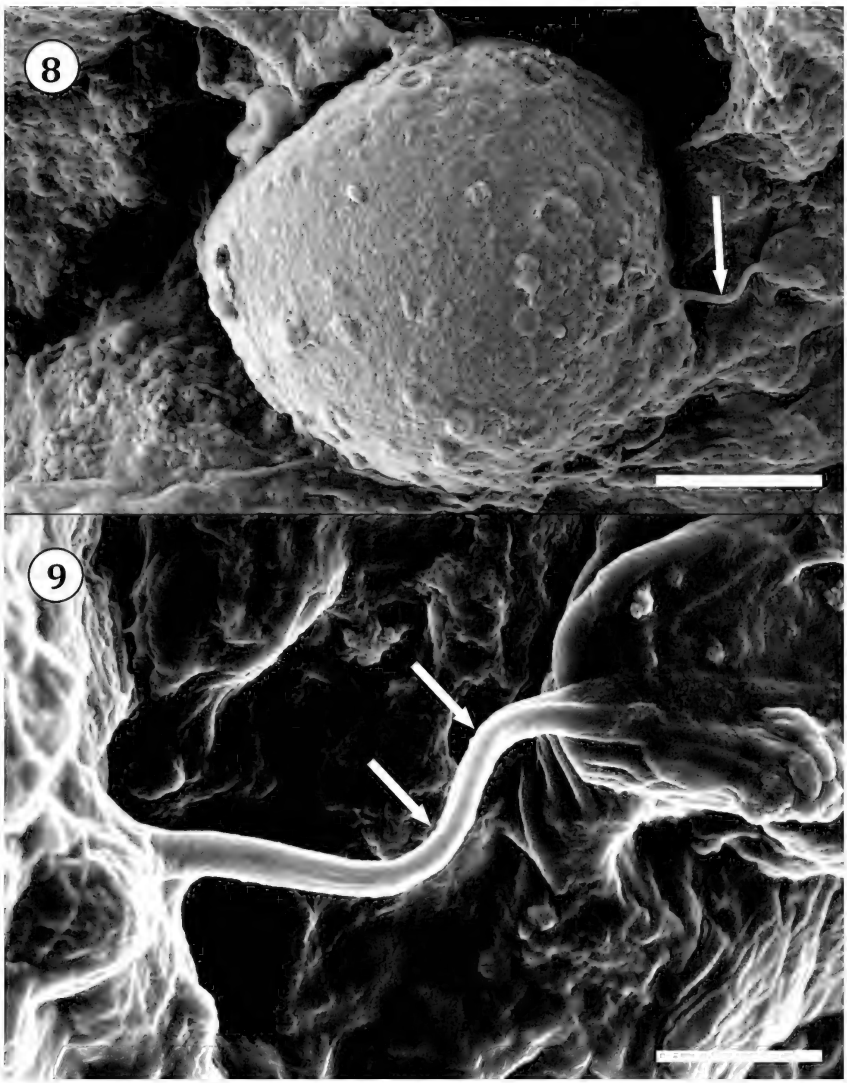
Etymology: *nyukfahii*, derived from the name of the second author's uncle, Nyuk Fah Lee, for his financial support of her studies at UTAR, Malaysia.

Colonies on natural substratum effuse, grayish, mycelium superficial and immersed; stroma none; setae and hyphopodia absent; aerial hyphae absent; conidiophores micronematous to semi-macronematous, mononematous, straight or flexuous, thin-walled and delicate, smooth, hyaline, 0–3-septate, up to 5 µm long and ca. 0.2–0.5 µm wide; conidiogenous cells monoblastic and polyblastic, integrated, terminal and intercalary, determinate, hyaline; conidia holoblastic, acropleurogenous, solitary, globose, 10–13 µm in diameter, one-



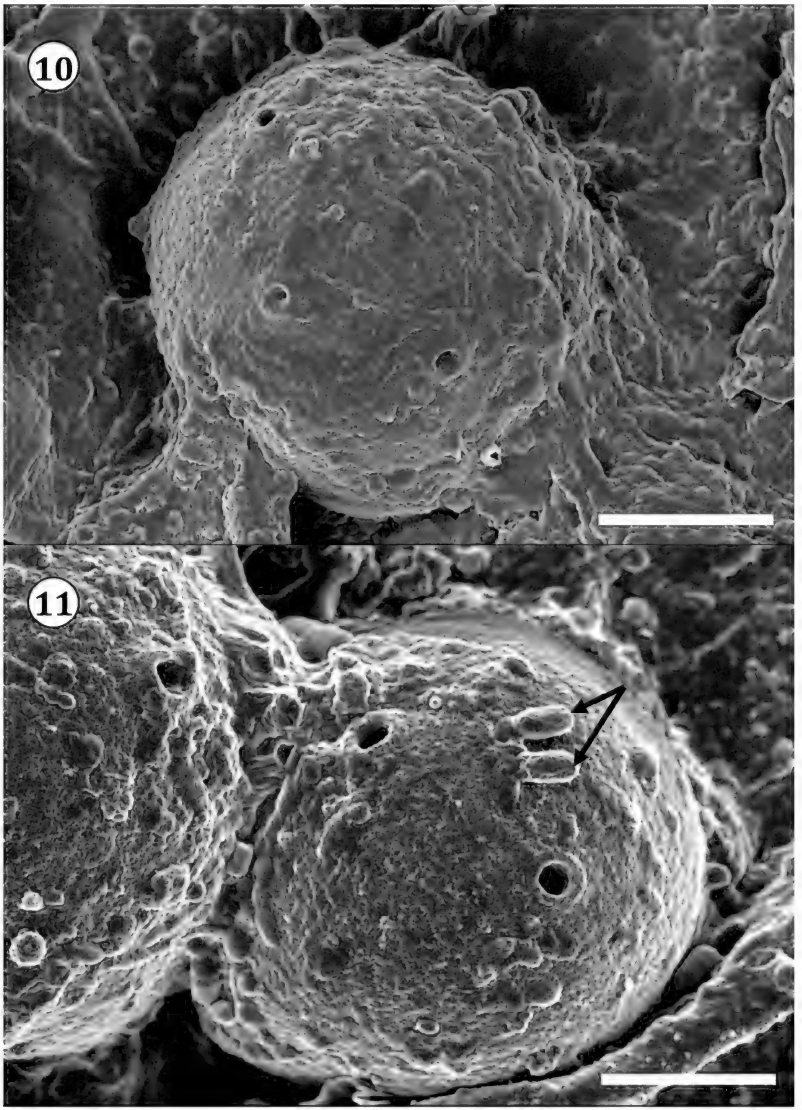
Figs 6–7. *Gilmaniella nyukfahii* (holotype, SEM). 6. Conidia on surface of natural substratum. 7. Conidial germ pore which is polygonal in surface view; the conidial wall is rugose in texture. Scale bars: 6 = 20 μm; 7 = 1 μm.

celled, thick-walled, slightly rugose or verrucose, yellowish or pale olivaceous brown when mature, with 5–7 distinct, somewhat protruding, polygonal germ pores which are ca. 0.5–0.8.5 μm diam.; conidial secession schizolytic.



Figs 8–9. *Gilmaniella nyukfahii* (holotype, SEM). 8. Conidium still attached to the delicate conidiophore (arrowed). 9. Conidiophore, which is smooth-walled and septate (arrowed). Scale bars: 8 = 5 µm; 9 = 1 µm.

Comments— Superficially, *Gilmaniella nyukfahii* resembles *Trichocladium ismailiense* Moustafa & Ezz-Eldin, which also produces dematiaceous conidia with several germ pores (Moustafa & Ezz-Eldin 1990, Goh & Hyde 1999). However, the conidial development in *Trichocladium* is holothallic (sensu Cole



Figs 10–11. *Gilmaniella nyukfahii* (holotype, SEM). 10. Conidium with multiple, somewhat protruding, distinct germ pores. 11. Conidia with germ pores; cocci and rod-shaped bacterial cells (arrowed) are visible clinging to the conidial surface. Scale bars = 5 μm.

& Samson 1979), whereas in *Gilmaniella* it is mono- or polyblastic. Additionally, in accepted species of *Trichocladium*, conidia are septate (Goh & Hyde 1999), whereas conidia of *Gilmaniella* species are unicellular (Umali et al. 1998).

Among the eight previously described *Gilmaniella* species, only *G. multiporosa* Moustafa & Ezz-Eldin is similar to *G. nyukfahii* in having conidia with more than one germ pore. However, *G. multiporosa* differs from our new species in several characters: habitat (soil), conidial shape (clavate to pyriform), conidial formation (clustered or catenate), and number of germ pores per conidium (1–4, mostly 2–3; Moustafa & Ezz-Eldin 1989).

In addition to producing conidia with 5–7 distinct germ pores, *G. nyukfahii* is also unique in its conidiophores, which are slim and delicate, and the details of which can be revealed only under the electron microscope.

Under the scanning electron microscope, the spore wall of *G. nyukfahii* appears slightly rugose and is coated with dung debris. Some bacterial cells (cocci and rods) adhering to the hyphae and spores are commonly observed in this habitat.

We have attempted unsuccessfully to grow *G. nyukfahii* in culture. At least 20 single spores of this species were isolated from its natural substratum using the method described by Goh (1999), but none germinated, whether on water or cow dung agar (Bills & Foster 2004). The failure of spore germination on artificial media might be due to the lack of certain environmental conditions or specific nutrient requirements for this particular species, which were not met in this study.

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Hygrocybe griseobrunnea, a new brown species from China

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Abstract — *Hygrocybe griseobrunnea*, a new species in *Hygrocybe* subsect. *Squamulosae*, is described and illustrated based on the morphological characters and molecular data. The fungus is characterized by numerous greyish brown to brown or dark brown squamules on the pileus surface, adnate to shortly decurrent lamellae, and a trichodermal pileipellis.

Key words — *Basidiomycetes*, *Hygrophoraceae*, taxonomy

Introduction

The genus *Hygrocybe* (Fr.) P. Kumm. (*Hygrophoraceae*, *Agaricales*, *Basidiomycota*) is distributed worldwide, with ~150 accepted species (Kirk et al. 2008) and ~670 proposed names (http://www.indexfungorum.org). *Hygrocybe* sect. *Squamulosae* (Bataille) Singer is characterized by the dry fruitbody, squamulose or tomentose pileus, smooth stipe, and trichodermal pileipellis (at least in the pileus centre) (Boertmann 2010). More than 15 species in subsect. *Squamulosae* have been reported from different parts of the world (Singer 1986, Arnolds 1995, Borgen & Senn-Irlet 1995, Desjardin & Hemmes 1997, Young & Wood 1997, Borgen & Arnolds 2004, Cantrell & Lodge 2004, Leelavathy et al. 2006, Boertmann 2010, Ronikier & Borgen 2010, Vrinda et al. 2013). Only three species of the subsection — *Hygrocybe cantharellus* (Schwein.) Murrill, *H. coccineocrenata* (P.D. Orton) M.M. Moser, and *H. turunda* (Fr.) P. Karst. — have been recorded from China (Zeng & Yang 1991, Bi et al. 1993, Shao & Xiang 1997, Chen & Li 2013).

Recently, a representative of subsect. *Squamulosae* was collected and studied during an investigation of the agaric flora in Chebaling National Nature Reserve, Guangdong province, China. Morphological examinations and molecular

analyses based on the internal transcribed spacer (ITS) sequences indicate that the fungus is distinct from any other known species of *Hygrocybe;* it is therefore described here as a new species.

Materials & methods

Specimens were photographed and annotated in the field and then dried in an electric oven. Macroscopic depictions were gained from the original field notes and photographs. The holotype is preserved in the Fungal Herbarium of the Guangdong Institute of Microbiology (GDGM). Colour descriptions are according to Kornerup & Wanscher (1978). Tissue sections were immersed in 5% potassium hydroxide (KOH) or 1% Congo Red for microscopical examination. From a mature specimen, 20 basidiospores and 10 basidia were randomly selected and measured in KOH. The notation (a–)b–c(–d) is used to describe basidiospore dimensions where the range b–c represents 90% or more of the measured values, and a and d are the extreme values. The length/width ratio of spores and basidia is presented as Q, and the mean ratio is presented as Q_m. Elements of immersed pileipellis, stipitipellis, and hymenophoral trama are also described.

Genomic DNA was extracted from the herbarium specimen using the Sangon Fungus Genomic DNA Extraction kit according to the manufacturer's instructions (Sangon Biotech Co., Ltd., Shanghai, China). The ITS region was amplified by PCR, using universal primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993). Amplified products were electrophoresed on 1% agarose gels with a known standard DNA marker and directly sequenced by Beijing Genomic Institute (BGI), and the holotype sequence was submitted to GenBank. These and GenBank reference sequences were used in phylogenetic analysis after being edited and aligned using Clustal 1.81 (Thompson et al. 1997) and MEGA5.1 (Tamura et al. 2011). The dataset was analyzed with maximum parsimony by PAUP* 4.0b10 (Swofford 2003) following He & Li (2013).

Taxonomy

Hygrocybe griseobrunnea T.H. Li & C.Q. Wang, **sp. nov.** Figs 1–2

MycoBank MB 804041

Differs from *Hygrocybe caespitosa* by its applanate pileus with a partly uplifted margin, longer basidia, and shorter spores and from *H. melleofusca* by its shorter spores and rarity of clamp connections in its hymenophoral trama.

Type: China, Guangdong Province, Chebaling National Nature Reserve, 27 August 2010, B. Song, C.S. Qiu & H. Huang (**Holotype**, GDGM 28755; Genbank, KC914883).

Etymology: from Latin, griseus = grey, brunneus = brown.

Pileus 16–32 mm broad, slightly convex when young, expanding to applanate with a partly uplifted margin, often cracked or even perforated in the centre with age, weakly striate from the margin to half way to the centre when mature, with greyish brown to brown or dark brown (6E3–5, 6F4–8) squamules on orange-grey to brownish grey (6B2, 6C2, 6D2) background, often with some very slight purple to violet (14A2, 15A2, 16A2) tint here and there, becoming paler grey (6B1, 6C1, 6D1) surface where the exfoliated scales have fallen off

Fig. 1. *Hygrocybe griseobrunnea* (holotype). Basidiomata in situ.

with age, usually fissile, and with violet-grey to greyish violet (18B2–3, 18C2–3) tint at margin. Lamellae adnate to short-decurrent, waxy, transparent, thick, fragile, up to 3 mm broad, concolorous at edge, white or near so, sometimes inconspicuously yellowish white (4A2 to 5A2), partially violet-white (18A2) especially near the pileus margin, with about 4 complete lamellae per cm, and usually with 1–3 lamellulae of different lengths between two complete lamellae. Stipe 12–28 × 3–6.5 mm (the length often shorter than the pileus diameter), central, hollow, terete or more often compressed terete, usually with a narrower base, in large fruitbodies irregularly furrowed, smooth, occasionally curved at base, white to pale violet or violet-grey to greyish violet (18A1–3, 18B2–3), becoming brownish orange to light brown (6C3, 6D4) at lower part when mature. Context thin, concolourous with the lamellae, fragile. Smell insignificant.

Basidiospores 6.0–8.5(–9.0) × 4.0–6.5(–7.0) μm, Q = 1.1–1.6, Q_m = 1.41, ellipsoid to oblong, smooth, thin walled, hyaline, inamyloid. Basidia narrowly clavate, 45–63 × 8–12 μm, Q = 4.9–5.6, Q_m = 5.2, thin-walled, with 2 or 4 sterigmata up to 8.0 μm long, with a basal clamp connection. Hymenophoral trama subregular, composed of hyaline, thin-walled, cylindrical elements (30–110 × 9–20 μm), sometimes inflated, and rarely with clamp connections. Pileipellis a trichoderm, made up of septate, thin-walled hyphae about 27–75 × 6–20 μm, with brown intracellular pigment, clamp connections usually absent; underlying hyphae repent, cylindrical, devoid of any pigments. Stipitipellis a cutis, with cells 2–7 μm broad.

Ecology & distribution — On soil, in broad-leaved forest, and so far known only from the type locality in China.

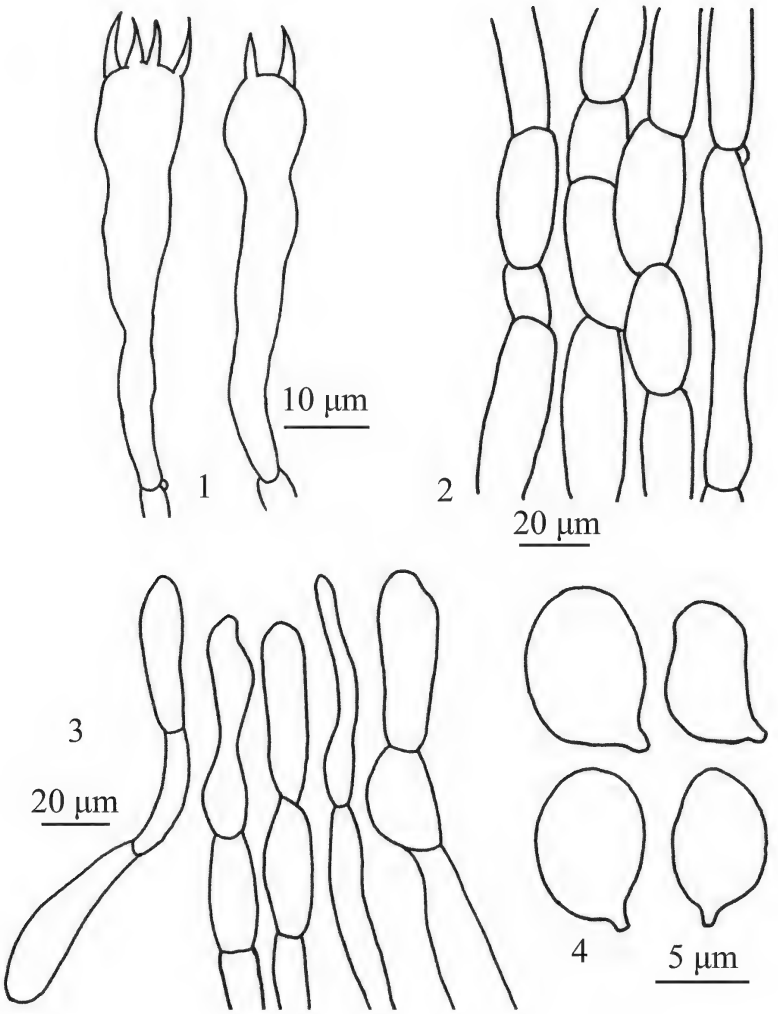

Fig. 2. *Hygrocybe griseobrunnea*.
1. Basidia. 2. Hymenophoral trama. 3. Elements of pileipellis. 4. Basidiospores.

Comments — *Hygrocybe griseobrunnea* is well characterized by its squamulose greyish brown to brown or dark brown pileus, subregular hymenophoral trama, and upturned pileipellis tufts. The trichodermal pileus squamules and subregular hymenophoral trama comprising hyphae <200 μm long place this fungus in *Hygrocybe* subsect. *Squamulosae* (Borgen & Arnolds 2004, Boertmann 2010).

Hygrocybe brunneosquamulosa Leelav. et al., described from India, is similar to *H. griseobrunnea* in sharing a grey-brown pileus with darker brown scales and sometimes having a central perforation in the mature pileus, but the two species are quite different because *H. brunneosquamulosa* has bright yellow, greyish brown or olive-brown lamellae, light yellow, amber-yellow or egg-yellow stipe, longer spores (7–10.5(–11) × 4.5–6.5(–7.5) μm), clamp-connections present in all parts of the basidioma, and numerous dark yellow-brown intracellular pigment in all tissues (Leelavathy et al. 2006; P. Manimohan, unpublished photo). *Hygrocybe caespitosa* Murrill, described from North America, also with a brownish squamulose pileus, is distinguished by its convex to obconic pileus with an inrolled margin, sinuate lamellae, yellow context, longer spores

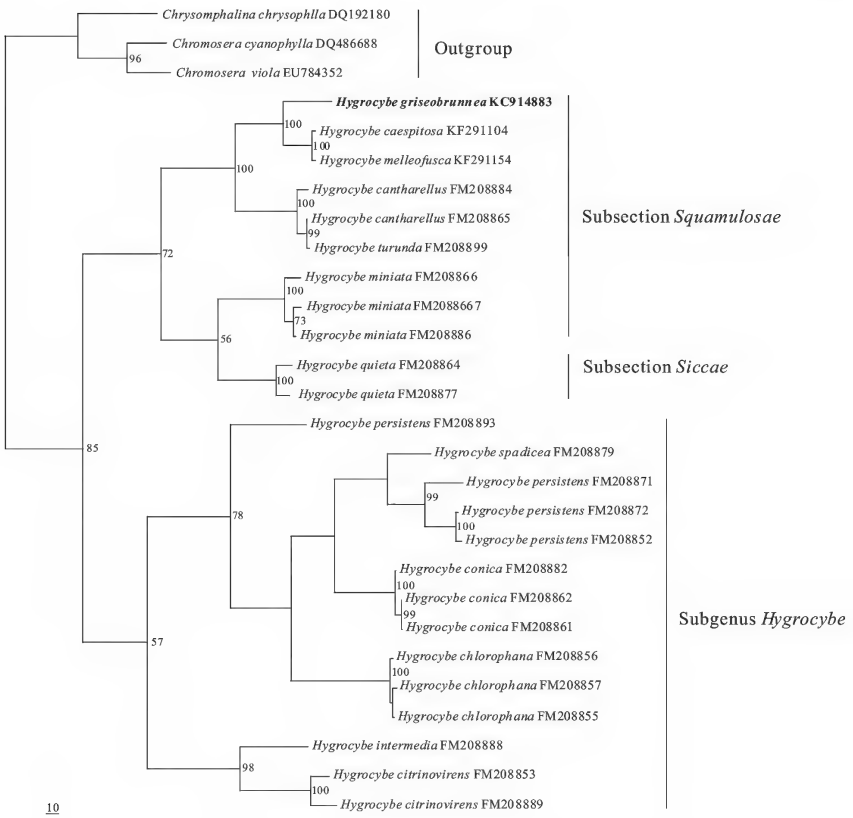

Fig. 3. The phylogenetic tree obtained from Maximum Parsimony analysis of ITS sequences of *Hygrocybe* species. *Chromosera cyanophylla*, *C. viola*, and *Chrysomphalina chrysophylla* were selected as outgroup. Parsimony bootstrap values (>50%) are shown. The unpublished ITS sequences of *H. caespitosa* and *H. melleofusca* were provided by Dr. D. Jean Lodge.

(7.5–10 × 5–6.5 μm), and smaller basidia (34.5–43 × 6–7.5 μm) (Murrill 1914, Cantrell & Lodge 2004). *Hygrocybe melleofusca* Lodge & Pegler, described from Puerto Rico, which resembles the new species with its dull coloured and often perforated pileus, has longer spores (7.8–10(–12) × 5–7(–8) μm) and prominent clamp-connections in the hymenophoral trama (Lodge & Pegler 1990).

The phylogenetic analysis (Fig. 3) clusters *H. griseobrunnea, H. caespitosa,* and *H. melleofusca* in the same clade with a 100% bootstrap value. This well-supported clade is sister to the clade with *H. turunda* (type species of subsect. *Squamulosae*). Bootstrap support for subsection *Squamulosae* (excluding *H. miniata*) is 100%, indicating that *H. griseobrunnea* belongs in the same subsection as *H. caespitosa* and *H. melleofusca*. Unfortunately, we were unable to obtain sequences from *H. brunneosquamulosa.* Furthermore, the ITS sequences of the *Hygrocybe* species formed two separated sister genetic groups that correspond to the two subgenera (*Hygrocybe* and *Pseudohygrocybe*) distinguished by morphological features (85% bootstrap support).

Acknowledgments

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Xylaria in southern China – 8. *X. bannaensis* sp. nov. and *X. brunneovinosa* new to the mainland

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Abstract—Two *Xylaria* species associated with termite nests in China are reported. *Xylaria bannaensis* is described as a species new to science, and *X. brunneovinosa* is reported for the first time from Mainland China. The morphological descriptions and photographs of stromata and microstructures are provided based on Chinese materials.

Key words—*Ascomycota*, *Xylariaceae*, *Xylariales*

Introduction

Many *Xylaria* species are frequently found emerging from termite nests, a long acknowledged association (Petch 1906). Many mycologists have been interested in the relationships between fungi and termites (Petch 1913, Lloyd 1918a,b, 1924, Dennis 1956, 1958, 1961, Boedijn 1959, Sands 1969, Heim 1977, Batra & Batra 1979, Rogers et al. 1987, Wood & Thomas 1989, Whalley 1996, Ju & Rogers 1999, Rogers et al. 2005, Ju & Hsieh 2008). *Xylaria* species are considered to function as either saprotrophs, inhabiting the comb material and competing with species of the agaric *Termitomyces* R. Heim for nutrients (Sands 1969, Heim 1977, Wood & Thomas 1989), or symbionts, making the comb material suitable for growth of both fungus and termite (Batra & Batra 1979). The diversity of termitophilous *Xylaria* species in China is still poorly known (Tai 1979, Abe & Liu 1995), and further investigations are highly desirable.

Materials & methods

Examined specimens are deposited in Herbarium of Mycology of Jilin Agricultural University (HMJAU). Microscopic features and measurements were made from slide

preparations mounted in water and Melzer's iodine reagent. The photographs of asci, ascal apical rings, and ascospores were taken by using a VHX-600E microscope (Keyence Corporation). The photographs of stromatal surface were taken with a ZSA30w microscope and S70 Canon camera.

Taxonomy

Xylaria bannaensis H.X. Ma, Lar.N. Vassilyeva & Yu Li, **sp. nov.** Fig. 1

MycoBank MB803942

Differs from *Xylaria arenicola* by its stromatal surface, cylindrical stromata and larger apical ring; and from *X. nigripes* by its larger ascospores.

Type: China, Yunnan Province: Mengla County, Xishuangbanna Tropical Botanical Garden, 21°43′N 101°26′E, alt. 500 m, on ground, 10 Aug 2010, Haixia Ma (**Holotype**, HMJAU 23549).

Etymology: Refers to the place where the fungus was collected.

Stromata unbranched, cylindrical, with rounded fertile apices, on long glabrous longitudinally wrinkled and deeply rooted stipes, 7–10 cm total length × 2.5–5 mm diam., externally dark brown to black, internally at first probably white, and eventually entirely black; texture very hard; surface wrinkled, somewhat cracked, roughened with perithecial mounds. Perithecia ovoid, 0.4–0.6 mm diam.; ostioles papillate. Asci eight-spored, cylindrical, long-stipitate, 60–90(–95) µm total length × 4–5.5 µm, the spore-bearing part 40–45 µm long, with apical ring bluing in Melzer's iodine reagent, minute, 1–1.3 µm high × 1–1.6 µm broad. Ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, smooth, (5.5–)6–7(–7.5) × 3–4 µm, with straight germ slit more or less spore-length.

Comments —*Xylaria bannaensis* resembles *X. arenicola* Welw. & Curr., according to the key of Ju & Hsieh (2008), but their stromatal surfaces are distinctly different. *Xylaria arenicola* has a rougher surface with protuberant perithecia, unlike the somewhat cracked and wrinkled surface of *X. bannaensis*. Compared to the distinctly cylindrical *X. bannaensis* stroma, that of *X. arenicola* is more flattened. Finally *X. arenicola* has a slightly smaller apical ring (both height and width are <1 µm; Rogers et al. 2005).

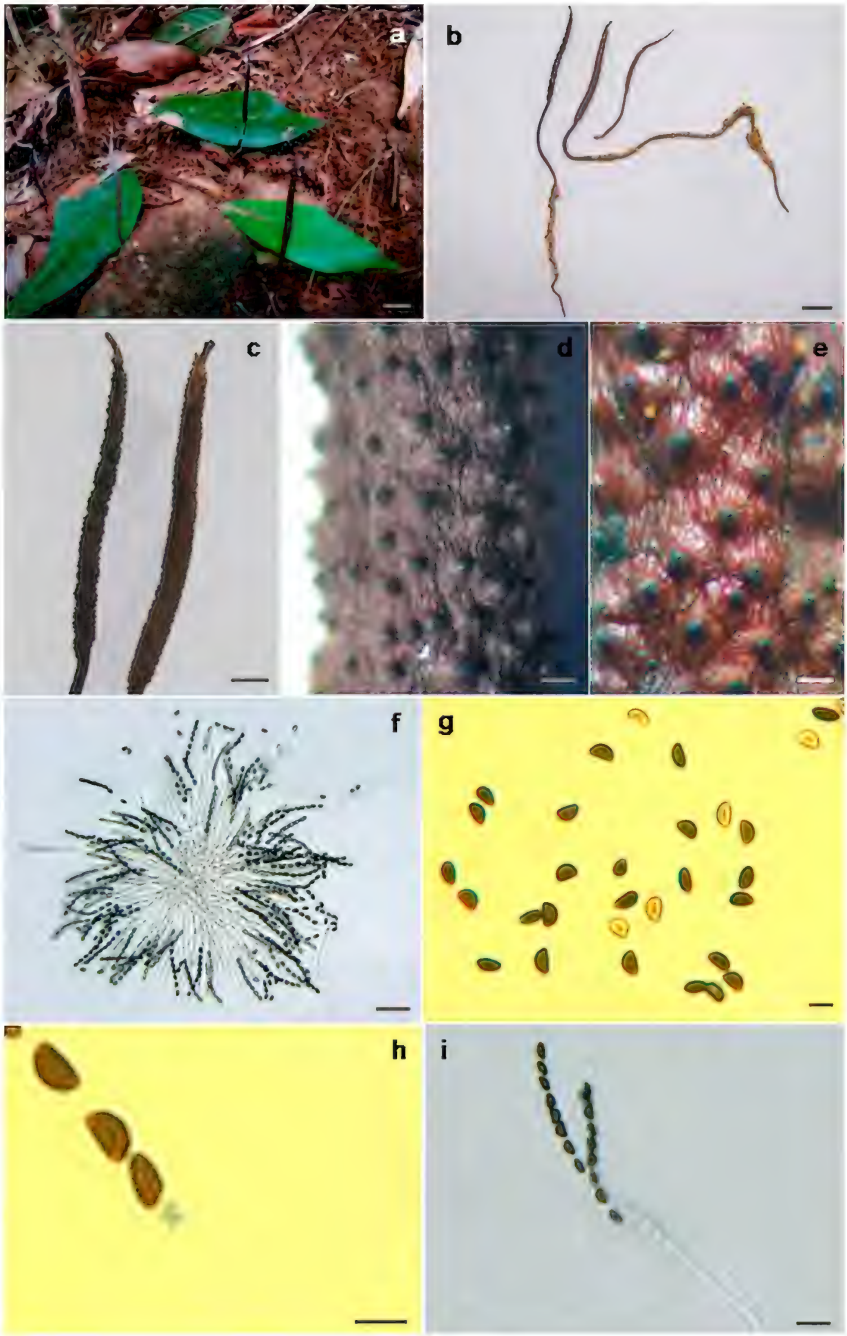
Xylaria arenicola was listed as a synonym (Dennis 1958) or a variety (Dennis 1961) of *X. nigripes* (Klotzsch) Cooke. *Xylaria bannaensis* resembles *X. nigripes* in stromatal morphology, but *X. nigripes* has smaller ascospores ((3.5–)4.5–5(–6) × 2–3 µm; Rogers et al. 2005).

Xylaria brunneovinosa Y.M. Ju & H.M. Hsieh, Mycologia 99(6): 941. 2008 ["2007"] Fig. 2

Stromata unbranched or sparsely branched from the base, long cylindrical to cylindric-clavate, sometimes flattened, with long sterile and acutely tapered

FIG. 1. *Xylaria bannaensis* (Holotype): a. Stromata; b. Fertile stromatal heads; c. Stromatal surface; d. Ascospores; e. Asci; f. Ascal apical ring (violet). Scale bars: a, b = 1 cm; c = 0.5 mm; d, e, f = 5 μm.

apices, on short or long, glabrous rooting black stipes, 3.5–7 cm total length × 1–5(–7) mm broad, the fertile parts gray brown or brown vinaceous, stipes black, interior at first white to cream, becoming ochraceous; texture very hard; surface smooth except for conspicuous perithecial contours. Perithecia globose, 0.3–0.4 mm diam., ostioles papillate. Asci eight-spored, cylindrical, long-stipitate, (80–)90–130(–140) μm total length × 4.5–5.5 μm broad, the spore-bearing part 43–55 μm long, with apical ring bluing in Melzer's iodine reagent, rectangular, 2–3 μm high × 1–2 μm broad. Ascospores brown to dark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brown, unicellular, ellipsoid-inequilateral, smooth, (5.8–)6–7(–7.5) × 4–5 μm, with straight germ slit slightly less than spore-length.

Specimens examined: **China, Yunnan Province:** Mengla County, Xishuangbanna Tropical Botanical Garden, 21°42′N 101°25′E, alt. 500 m, on ground, 10 Aug 2010, Haixia Ma (HMJAU 23577, 23578, 23579).

Comments — *Xylaria brunneovinosa* is easily recognizable by its narrow cylindrical stromata and its stromatal exterior and interior colors. This species, previously reported only from Indonesia and Taiwan, is reported for the first time for Mainland China. The Chinese specimens are quite similar to *X. piperiformis* Berk. and *X. cirrata* Pat. in stromatal morphology and ascospore size, but *X. brunneovinosa* can be easily separated by its brown vinaceous exterior and ochraceous interior. The ascospores of *X. cirrata* are usually minutely pinched at the ends (Ju & Hsieh 2008). *Xylaria piperiformis,* described from Indian material (Rogers et al. 2005), has a smaller apical ring (cuboid, 1.5 μm), the shorter asci (44 μm total length × 4.4 μm broad), and shorter spore-bearing part of asci (ca. 36 μm).

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Fig. 2. *Xylaria brunneovinosa* (HMJAU 23578): a, b. Stromata; c. Fertile parts of stromata; d, e. Stromatal surface; f, i. Asci; g. Ascospores; h. Ascal apical ring (violet). Scale bars: a, b = 1 cm; c = 5 mm; d = 0.5 mm; e = 0.2 mm; f = 30 μm; g, h = 5 μm; i = 10 μm.

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A new species of *Scutellinia* from Mexico

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Abstract — *Scutellinia ulloae* sp. nov., collected on soil from Temascaltepec in central Mexico near Nevado de Toluca (aka Xinantécatl) volcano, is described and illustrated. A unique tuberculate ascospore ornamentation distinguishes the new species from the approximately 60 other known *Scutellinia* taxa.

Key words — *Pezizales*, biological diversity, Neovolcanic Axis

Introduction

In his *Scutellinia* monograph, Schumacher (1990) recognized 45 species and 2 subgenera: *Scutellinia* and *Legalia.* More recent descriptions of 16 new taxa — *S. ahmadiopsis* (Zhuang 2005b), *S. alleghenensis* (Moravec 1989), *S. beijingensis* (Zhuang 2005b), *S. citrina* (Yao & Spooner 1995), *S. crinita* var. *discreta* (Matočec et al. 2005), *S. fujianensis* (Cao & Moravec 1989), *S. jejuensis* (Han et al. 2010), *S. jilinensis* (Yu et al. 2000), *S. kerguelensis* var. *microspora* (Zhuang 2005b), *S. korfiana* (Zhuang 2005a), *S. laevispora* (Moravec 1997), *S. orientalis* (Choi et al. 2013), *S. sinensis* (Liu & Peng 1996), *S. sinosetosa* (Zhuang & Wang 1998), *S. totaranuiensis* (Moravec 1996), and *S. tuberculata* (Matočec 2000) — have brought the world total to approximately 60 recognized species.

With a surface area of two million km^2, Mexico is one of world's five most megadiverse countries, encompassing 10% of the world's biodiversity (Sarukhán 1995). Guzmán (1998) has estimated the number of fungal species in Mexico at close to 200,000. In Mexico, six species of *Scutellinia* have been recorded:

S. asperrima (Seaver) Le Gal, *S. cubensis* (Berk. & M.A. Curtis) Gamundí, *S. scutellata* (L.) Lambotte, *S. setosa* (Nees) Kuntze, *S. trechispora* (Berk. & Broome) Lambotte, and *S. umbrorum* (Fr.) Lambotte (Denison 1959; Vázquez del Mercado 1977; Welden et al. 1979; Frutis & Guzmán 1983; Chacón & Guzmán 1984; Guzmán-Dávalos & Trujillo-Flores 1984; Portugal et al. 1985; Bautista et al. 1986; Medel & Chacón 1988; Téllez-Bañuelos et al. 1988; Esqueda-Valle et al. 1992; Pompa-González & Cifuentes 1991; Montañez 1999; Valenzuela et al. 2004; Chanona-Gómez et al. 2007). We describe below another new species originally collected in 1988 from the southern part of the Neovolcanic Axis belonging to Estado de Mexico (Ferrusquía-Villafranca 1993).

Materials & methods

The only specimen of our new species (with three apothecia) was examined using standard mycological techniques (Schumacher 1990; Cifuentes et al. 1986; Izquierdo-San Agustín 2008) and deposited in the Herbarium FCME. The type specimen was prepared for scanning electron microscopy according to the methods described by Zhuang (2005b). Colors are from Methuen Handbook of Colour (Kornerup & Wanscher 1978). We attempted to extract DNA for molecular analysis. One apothecium was used in this process and no electrophoretic bands were detected even after PCR amplification. We decided not to sacrifice more apothecia.

Taxonomy

Scutellinia ulloae L. Izquierdo, S. Sierra, Rodr.-Gut., C.-Santiuste & Cifuentes, **sp. nov.** FIG. 1

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Differs from *Scutellinia chiangmaiensis* by a spore ornamentation of cylindrical to obpyriform tubercles that are slightly constricted at the base and slightly connected by small ridges that do not form a true reticulum.

TYPE — Mexico, Estado de Mexico, Mpio. de Temascaltepec, km 71 carretera Toluca–Tejupilco, desviación a Presa Chica (19°02′14″N 100°03′00″W), alt. 1950 m, on soil, ground, 23 IX 1988, Hilario y Villegas 1130 (**Holotype**, FCME 14557).

ETYMOLOGY — In honor to the eminent Mexican mycologist Dr. Miguel Ulloa, who has contributed extensively to the knowledge of Mexican ascomycete biota.

APOTHECIA discoid, sessile, 1–4 mm in diam., hymenium surface reddish orange (Methuen 7A8), receptacle (external surface) off-white, side straight and cartilaginous consistency, covered with brown hairs; MARGINAL HAIRS 648–1224 × 18–35(–41) µm, wall 3–3.5 mm, brown, unbranched, non-septate, although there are bifurcate bases, there is no difference between marginal hairs and lateral hairs; ECTAL EXCIPULUM of subangular, isodiametrical, hyaline cells, with slightly thickened walls, 40–45 µm in diam.; MEDULLARY EXCIPULUM composed of broad and short angular cells, 9–12 µm in diam.; ASCI

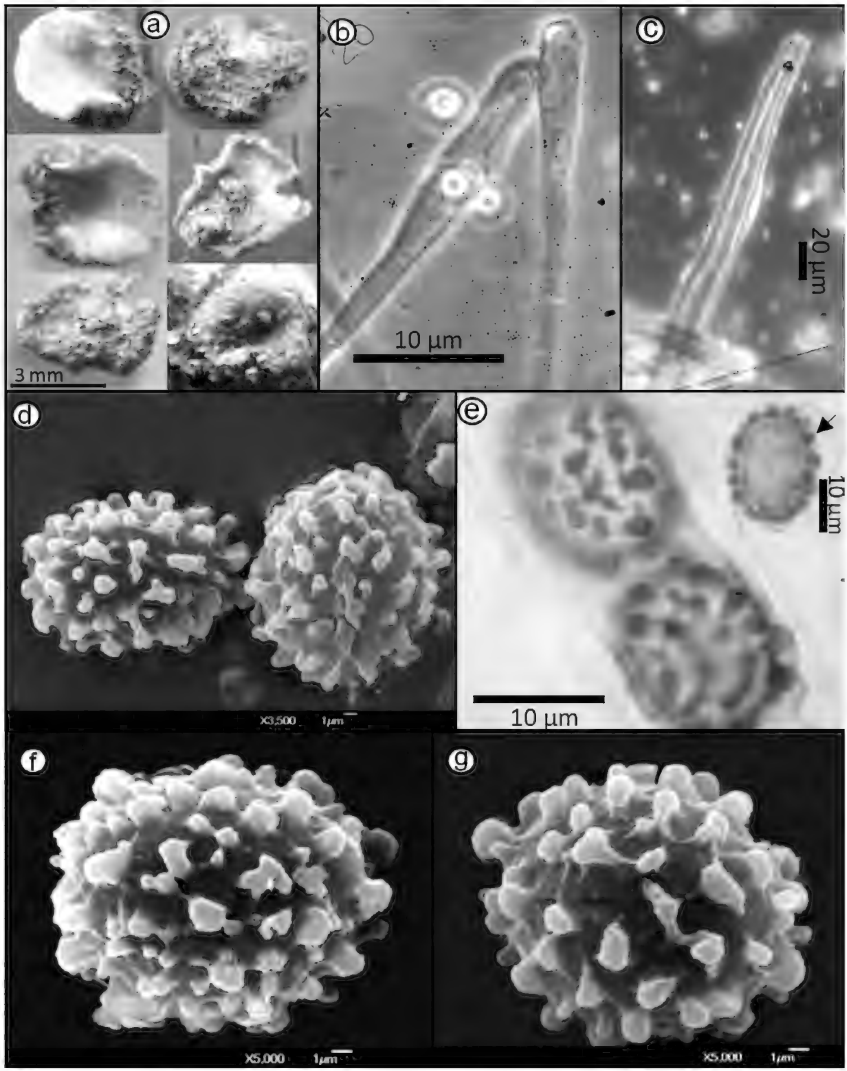

FIGURE 1. *Scutellinia ulloae* (holotype, FCME 14557). (a) Dried apothecial specimens; (b) paraphyses; (c) marginal hair; (d, f, g) ascospores in SEM; (e) ascospores in LM (phase contrast; arrow shows spore wall detail).

8-spored, operculate, cylindrical, 240–272 × 15.6–31.2 µm wide, inamyloid; ASCOSPORES ellipsoid (15–)17–17.5 × 12–13(–14) µm, Q = 1.38, with one guttule to multiguttulate, SPORE ORNAMENTATION: tubercles 2–2.5 µm high ×

2 µm broad, cylindrical to obpyriform, slightly constricted at the base, some interconnected by slim line when viewed by SEM, spore ornamentation when stained with cotton blue easily visible in light microscopy. PARAPHYSES slender, 2–3 µm wide, clavate and 5.6–8.4 µm wide at the apex, septate, exceeding asci by 15–19 µm.

Discussion

Following Schumacher (1990), the species closest to *Scutellinia ulloae* is *S. chiangmaiensis* T. Schumach.; however, the spore ornamentation differs significantly. In *S. chiangmaiensis* the ornamentation is tuberculo-reticulate, giving an alveolate spore surface, while in *S. ulloae* tubercles are cylindrical to obpyriform, slightly constricted at the base and are slightly connected by small ridges that do not form a true reticulum. The vegetation type is mixed deciduous forest for *S. chiangmaiensis* and coniferous forest for *S. ulloae*.

The type specimen (FCME 14557) was initially misdetermined by Pompa-González & Cifuentes (1991) as *S. diaboli* (Velen.) Le Gal (= *S. trechispora*). The record of *S. trechispora* in Mexico is no longer correct and should be deleted.

Acknowledgments

We wish to thank Dr. Wen-Ying Zhuang (Institute of Microbiology, Chinese Academy of Sciences), Dr. Richard P. Korf (Cornell University, Ithaca, NY), and Dr. Santiago Chacón Zapata (Instituto de Ecología, Xalapa, Mexico) for reviewing the manuscript and for helpful comments; Dr. Jae-Gu Han for molecular analysis; and Bióls. Lilia Pérez-Ramírez (FCME Herbaria, Fac. de Ciencias, UNAM) for curatorial support. The SEM photographs were made in the Laboratorio de Microscopia de Barrido, Fac. de Ciencias, UNAM by Dr. Silvia Espinosa Matias. This research was supported by DGAPA PAPIIT IN–223704, 209605, 218008, 207311.

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Study on the phylogeny of *Nephroma helveticum* and allied species

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Abstract — *Nephroma subhelveticum* sp. nov. is described, supported by both morphological and molecular data as a species new to science. The nrDNA ITS sequence analysis indicates that *N. subhelveticum* and *N. flavorhizinatum* are allied species of *N. helveticum*, and that *N. isidiosum* derives from *N. helveticum*.

Key words — Asia, China, evolution, lichen, taxonomy

Introduction

The species of *Nephroma* Ach. (*Nephromataceae*) have middle-sized foliose thalli, cyanobacteria (sometimes green algae) as their photobionts, apothecia produced on the lower surface, 8-spored asci, commonly 3-septate ascospores, and often abundant terpenoids (James & White 1987, Burgaz & Martínez 1999, Brodo et al. 2001, Wetmore & Nash 2002, Louwhoff 2009). This genus includes 37 species worldwide (Tian et al. 2011).

Both *Nephroma helveticum* and *N. isidiosum* are cosmopolitan species with terpenoids, and only these two species in the genus have isidia (Brodo et al. 2001). *Nephroma helveticum* has lobules and flat isidia, while *N. isidiosum* has true cylindrical or branched isidia (Brodo et al. 2001). *Nephroma flavorhizinatum* also has lobules and terpenoids (Tian et al. 2011). The similar morphological and chemical characters indicate that these three species may be closely related. Recently some morphologically similar specimens that also have terpenoids, lobules, and very crisp thalli, were found from China. The nrDNA ITS sequences generated from these *Nephroma* representatives have been analysed so as to establish their phylogenetic relationships.

Table 1. GenBank sequences of *Nephroma* and *Lobaria* included in the molecular analyses.

Species	Origin	GenBank no.
N. helveticum	China, Yunnan	DQ001292
	Macaronesia	HQ455071
	USA, southwest	AY124119
	China, Tibet	AY124120
	USA, northwest	AY124124
N. subhelveticum (recorded as *N. helveticum*)	South Korea	DQ066705
	South Korea	DQ066706
	China, central	AY124118
	China, central	AY124122
	China, northeast	AY124127
	Russia, northwest	AY124128
N. parile	Canada, east	AY124148
N. resupinatum	South Korea	DQ066710
L. macaronesica	Outgroup	GU072745
L. pulmonaria		GU072753
L. retigera		EU626996

Materials & methods

Morphology & chemistry

The specimens studied are preserved in SDNU (Lichen Section of Botanical Herbarium, Shandong Normal University). The morphology and anatomy of the specimens were examined using an Olympus SZX16 stereomicroscope and Olympus BX61 compound microscope; morphological characters were photographed under Olympus with DP72. Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with C system (Orange et al. 2010).

Molecular analyses

Taxon sampling — nrDNA ITS sequences were obtained from 41 Chinese *Nephroma* specimens; 13 additional *Nephroma* sequences and outgroup sequences from three *Lobaria* species were downloaded from GenBank (Table 1).

PCR amplification and sequencing — Total DNA was extracted by the modified CTAB method (Rogers & Bendich 1988). E9 (ttgtacacaccgcccgt), NF1R (atccgaggtcaatcgtgt), NF2R (tgatccgaggtcaatcgt), and CL2R (tttctttcctccgcttattga) were used for PCR amplification of the nrDNA ITS as primers. PCR reaction program: initial denaturation at 95°C for 3 min, followed by 35 cycles of 30 s denaturation at 94°C, 45 s annealing at 58°C, 1 min extension at 72°C, and completed with a final 8 min extension at 72°C. Products were purified with Gel Extraction Mini Kit (SABC). Shanghai Genecore Corp. carried out the sequencing reactions.

Data analysis — The sequences were aligned using MAFFT version 7 (Katoh & Standley 2013). The aligned ITS matrix was edited manually and phylogenetic analyses were conducted using MEGA 5 (Tamura et al. 2011). The phylogenetic tree was inferred using the Minimum Evolution method (Rzhetsky & Nei 1992) and tested by 1000 bootstrap replications (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). All positions containing

alignment gaps and missing data were eliminated in pairwise sequence comparisons (Pairwise deletion option). A total of 606 positions comprised the final dataset.

Morphology & chemistry

Nephroma helveticum Ach., Lich. univ.: 523 (1810) FIGS 1, 2

Thallus foliose, leathery. Lobes with stout lobules (0.5 × 1 mm) at margin. Upper surface brown; isidia and soredia absent; tomentum and pruina often present; lobules marginal and laminal, often branched. Upper cortex pale

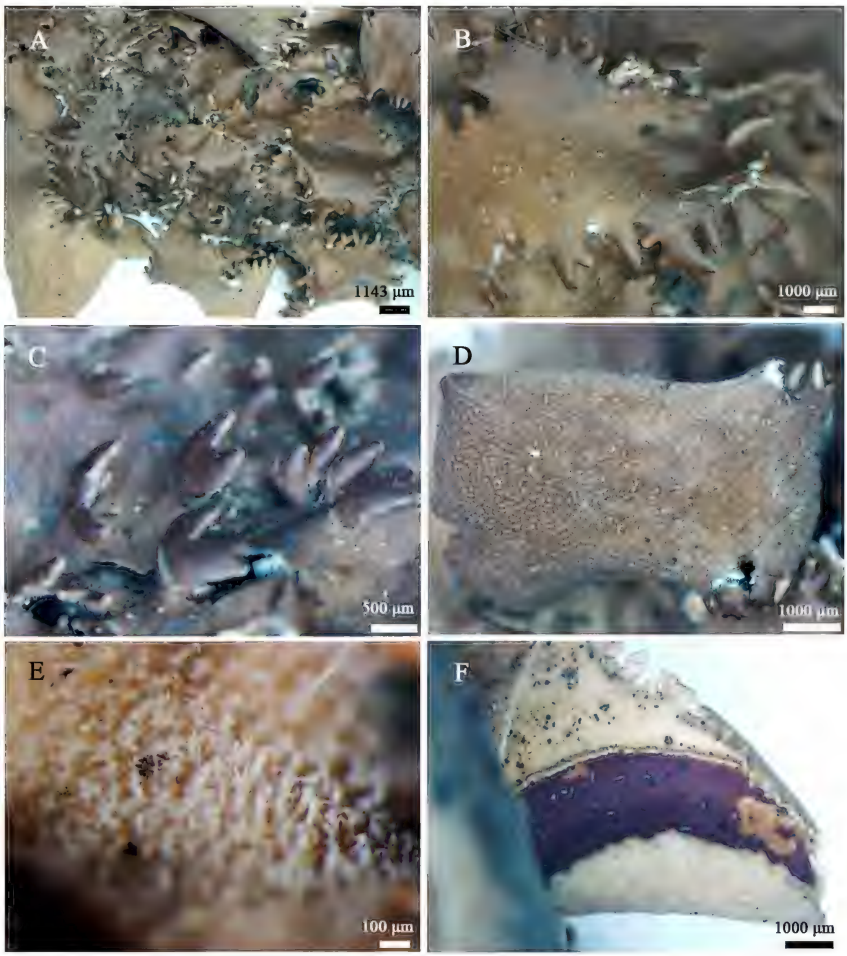

FIGURE 1. *Nephroma helveticum* (20080653). A: Thallus; B: Lobe with marginal lobules; C: Laminal lobules; D: Rough and tomentose dorsal surface of apothecium; E: Tomentum on lower surface; F: Apothecium immersed on lower surface.

brown, ca. 35 μm thick; photobiont *Nostoc*, ca. 65 μm thick; medulla white, with abundant crystals soluble in water, ca. 80 μm thick; lower cortex pale, ca. 30 μm thick. Lower surface brown, tomentose, paler and smooth at the margins; rhizines stubby, sparse. Apothecia common, immersed on lower surface at tips of lobes, kidney-shaped; disc dark brown, ≤7 mm in diameter; dorsal surface rough, with abundant clustered tomentum; margin usually crenulate. Epihymenium pale brown, without crystals, ca. 15 μm tall; hymenium hyaline, without crystals, ca. 40 μm tall; hypothecium pale brown, without crystals, ca. 35 μm tall. Paraphyses simple. Asci clavate, I+ blue, 8-spored; ascospores pale brown, 3-septate, 15–20 × 3–4 μm. Pycnidia not seen.

Spot tests — Thallus upper surface: K–, C–, KC–, PD–; medulla: K–, C–, KC–, PD–.

Secondary metabolites — N1, N2, ± other lichen substances (Fig. 3).

Ecology & distribution in China— on bark or moss in Guizhou, Jilin, Shanxi, Shaanxi, Sichuan, Tibet, and Yunnan.

Specimens examined — **CHINA. Guizhou**, Kaili, Mt. Leigong, alt. 1027 m, on bark, 2 Nov 2009, H.Y. Wang 20102726. **Jilin**, Wangqing, Mt. Tulaopoding, alt. 1100 m, on moss, 22 Jul 2012, H.Y. Wang 20129292. **Shanxi**, Ningwu, Mt. Luya, alt. 2500 m, on bark, 26 Aug 2011, H.Y. Wang 20121819A. **Shaanxi**, Meixian, Mt. Taibai, alt. 2100 m, on bark, 2 Aug 2005, W. Fu 20083001; 17 Jun 2011, Z.L. Huang 20114881(GenBank JX867681); alt. 2150 m, on bark, 17 Jun 2011, Z.L. Huang 20114339 (GenBank JX867682); alt. 2200 m, on bark, 2 Aug 2005, W. Fu 20080332, 20100571; alt. 2700 m, on bark, 17 Jun 2011, Z.L Huang 20114639K; alt. 2950 m, on bark, 17 Jun 2011, Z.L. Huang 20114717 (GenBank JX867703). **Sichuan**, Litang, alt. 4200 m, on bark, 5 Nov 2008, H.Y. Wang 20080388; Litang, Mt. Kazila, alt. 4700 m, on bark, 7 Nov 2008, H.Y. Wang 20080323, 20080354. **Tibet**, Nyingchi, Dongjiuxiang, alt. 2550 m, on bark, 17 Jul 2011, Y.L. Cheng 20114928, 20117991 (GenBank JX867707), 20117991A (GenBank JX867710), 20117991C (GenBank JX867713), 20118005, 20118005A, 20118005D, 20118005E, 20118005F (GenBank JX867712), 20118006B (GenBank JX867704), 20118006C (GenBank JX867683), 20118011, 20118044, 20118255A (GenBank JX867709), 20118300 (GenBank JX867705), 20118545; Mt Sejila, alt. 4100 m, on bark, 20 Oct 2007, G.Y. Han 20072907; alt. 4500 m, on bark, 20 Jul 2011, Y.L. Cheng 20118118A, 20118461, 20118729A (GenBank JX867706), 20118729E; Lulangzhen, observation station, alt. 2550 m, on bark, 14 Jul 2011, D.F. Jiang 20119099. **Yunnan**, Shangri-La, Tianshengqiao, alt. 3500 m, on bark, 3 Nov 2008, H.Y. Wang 20080653 (GenBank JX867708), 20081370, 20081391, 20081515, 20081715, 20081885, 20082058, 20082065, 20082181, 20082185, 20082213, 20082264, 20082270, 20082291, 20082355, 20102930, 20083063, 20083097, 20083312, 20083385, 20083861, 20086001, 20086011; Mt. Shika, alt. 4300 m, on bark, 2 Nov 2008, H.Y. Wang 20081991, 20082033, 20082218, 20083336, 20086017; Yulong, Mt. Laojun, alt. 4000 m, on bark, 6 Nov 2009, H.Y. Wang 20100564 (GenBank JX867711); 7 Nov 2009, H.Y. Wang 20090001, 20100562; Jianchuan, Mt. Shibao, alt. 2600 m, on bark, 31 Oct 2008, H.Y. Wang 20083409; Lijiang, Mt. Yulong, alt. 3200 m, on bark, 7 Nov 2009, M. Li 20102729A.

Comments — *Nephroma helveticum* is considered to produce flat isidia (Brodo et al. 2001). However, we did not find true isidia on our specimens. The lobules of *N. helveticum* from China are varied (Fig. 2 I–N), sometimes very narrow and

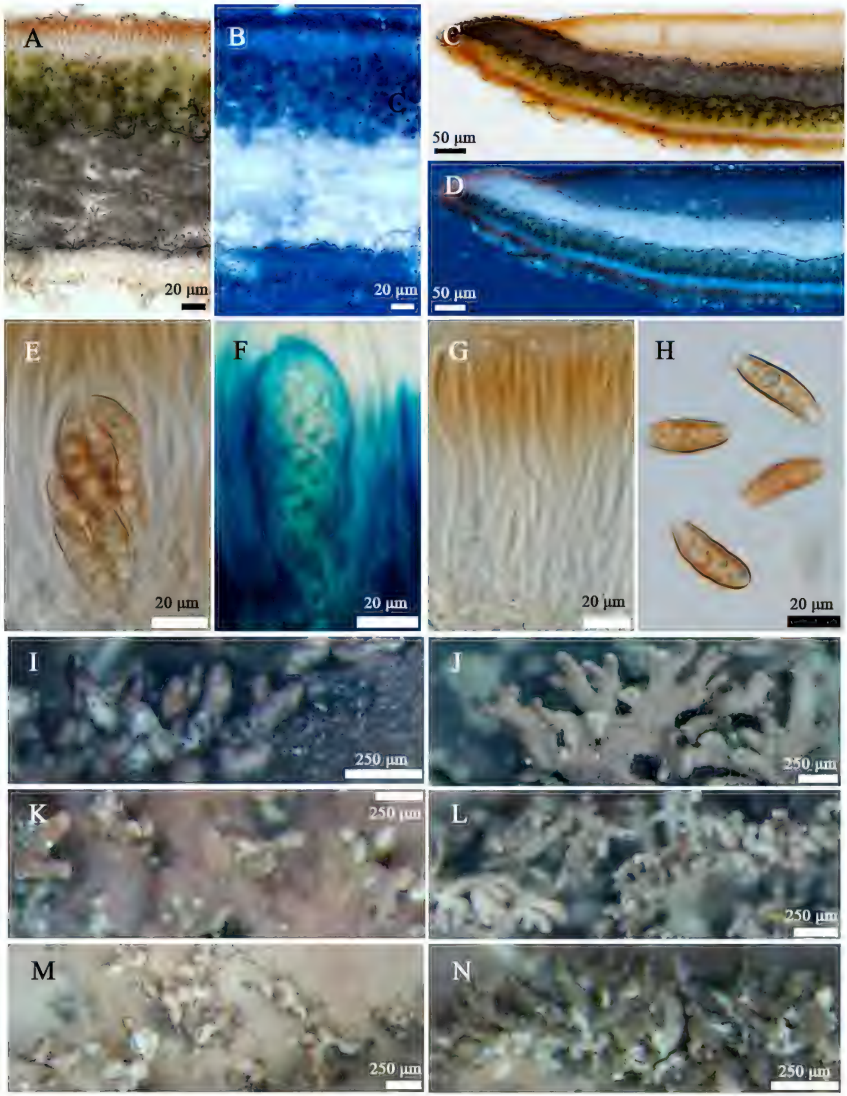

FIGURE 2. *Nephroma helveticum* (A–H, 20080653; I, 20080323; J, 20118011; K, 20118005D; L, 20118729E; M, 20080388; N, 20100564). A: Section of thallus; B: Crystals in thallus; C: Section of apothecium; D: Crystals in apothecium; E: Ascus and ascospores; F: Iodine reaction of ascus and paraphyses; G: Paraphyses; H: Ascospores; I–N: Various laminal lobules.

isidium-like but always dorsiventral. Although *N. helveticum* and *N. isidiosum* have similar secondary substances (TLC, FIG. 3), *N. isidiosum* can be clearly separated by its typical cylindrical to coralloid isidia and lack of apothecia.

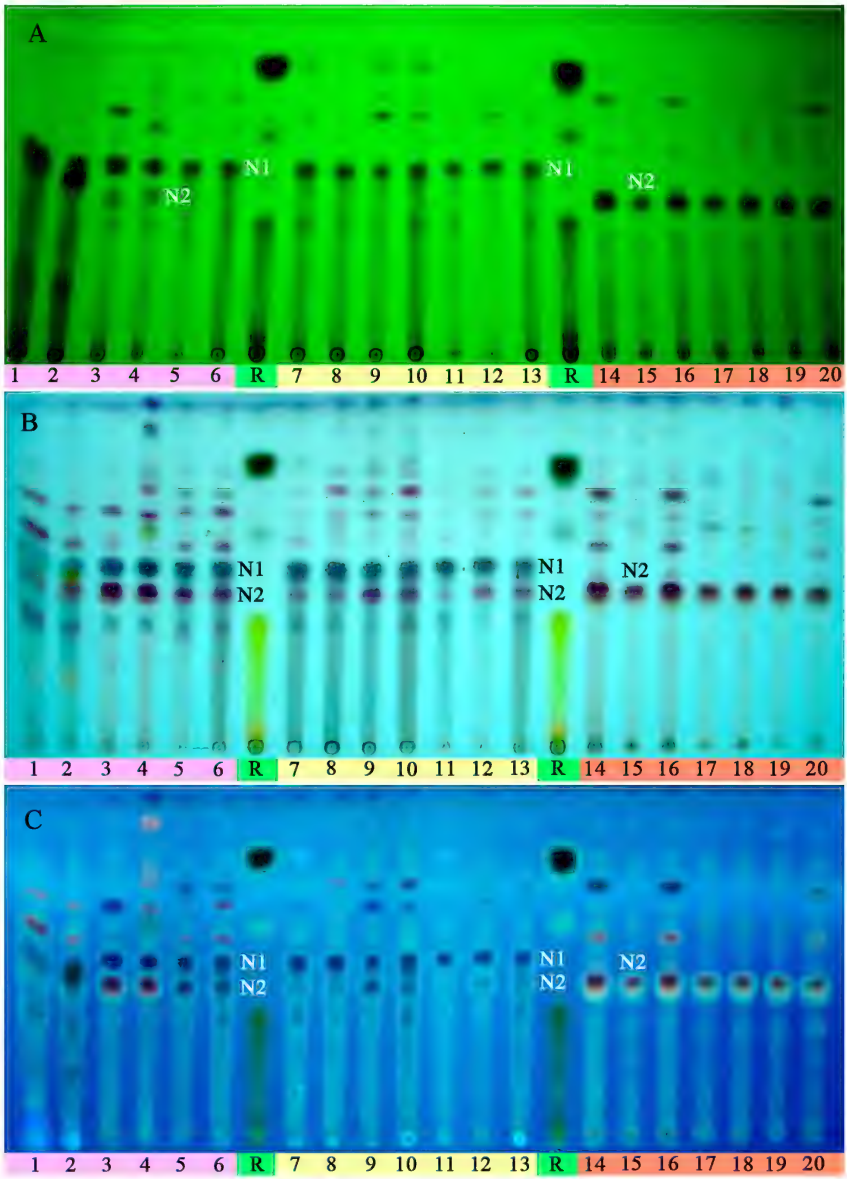

FIGURE 3. TLC results: A: UV254 nm before acid; B: Sunlight after acid and heating; C: UV365 nm after acid and heating. *Nephroma helveticum* (1, 20100564; 2, 20114717; 3, 20118255A; 4, 20117991C; 5, 20118300; 6, 20080653); *N. isidiosum* (7, 20080192; 8, 20081753; 9, 20084006; 10, 20083227; 11, 20084002; 12, 20114639D; 13, 20081280); *N. subhelveticum* (14, 20108511; 15, 20106583; 16, 20108510; 17, 20106494; 18, 20106581; 19, 20106595; 20, 20105971).

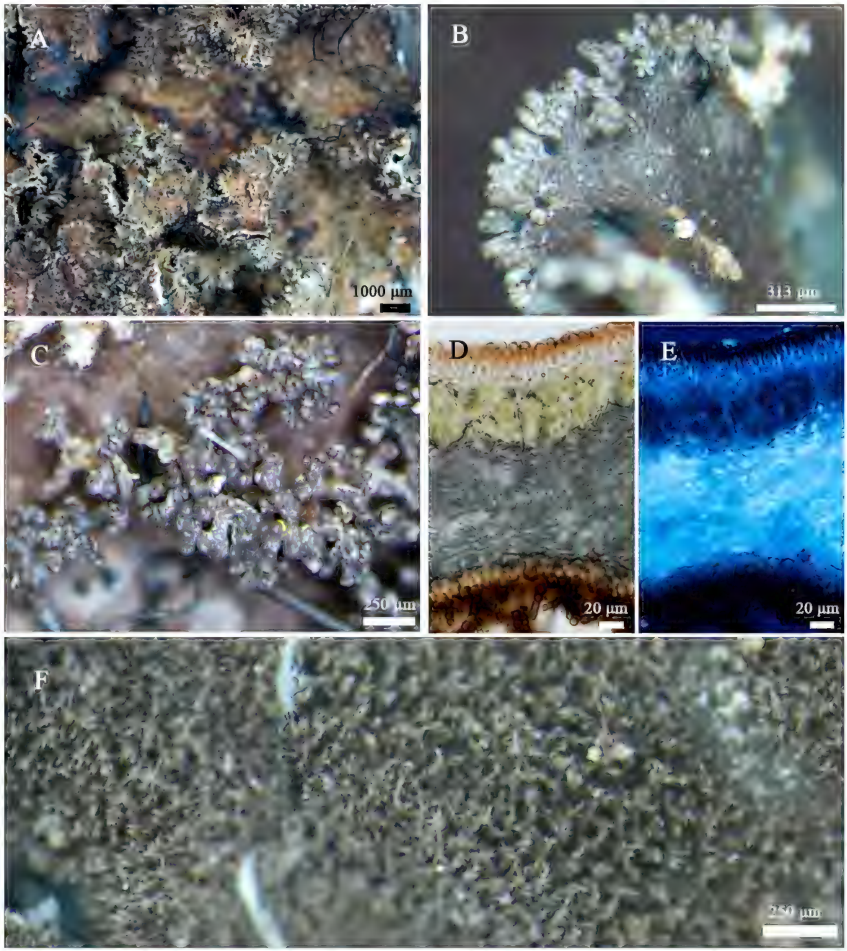

FIGURE 4. *Nephroma isidiosum* (20080192). A: Thallus; B: Lobe with marginal isidia; C: Laminal isidia; D: Section of thallus; E: Crystals in thallus; F: Tomentum on lower surface.

Nephroma isidiosum (Nyl.) Gyeln., Annals Cryptog. Exot. 4: 126 (1931) FIG. 4

Thallus foliose, leathery. Lobes with cylindrical isidia at margin. Upper surface brown; lobules and soredia absent; tomentum and pruina often present; isidia marginal and laminal, cylindrical to coralloid. Upper cortex pale brown, ca. 30 µm thick; photobiont *Nostoc*, ca. 50 µm thick; medulla white, with abundant crystals soluble in water, ca. 90 µm thick; lower cortex brown, ca. 12 µm thick. Lower surface brown, tomentose, paler and smooth at the margins; rhizines stubby, sparse. Apothecia and pycnidia not seen.

Spot tests—Thallus upper surface: K–, C–, KC–, PD–; medulla: K–, C–, KC–, PD–.

Secondary metabolites — N1, N2, ± other lichen substances (Fig. 3)

Ecology & distribution in China — on bark or ground with moss in Shaanxi, Sichuan, Tibet, and Yunnan.

Specimens examined — **CHINA. Shaanxi**, Meixian, Mt. Taibai, alt. 2700 m, on bark, 17 Jun 2011, Z.L. Huang 20114639D (GenBank JX867700). **Sichuan**, Litang, alt. 4200 m, terricolous, 5 Nov 2008, Z.S. Sun 20080225, 20080345, 20080753, 20080843, 20081280 (GenBank JX867701), 20084000, 20084006 (GenBank JX867689), 20084008 (GenBank JX867698); on bark, 5 Nov 2008, Z.S. Sun 20080661 (GenBank JX867694), 20083995, 20084004 (GenBank JX867693); 7 Nov 2008, H.Y. Wang 20084003 (GenBank JX867690); Mt. Kazila, alt. 4700 m, 7 Nov 2008, on bark, H.Y. Wang 20080238 (GenBank JX867695), 20080286, 20080769, 20080871, 20080865, 20081923 (GenBank JX867697), 20082001 (GenBank JX867687), 20084002 (GenBank JX867696), 20084005, 20084009, 20084010, Z.S. Sun 20080192 (GenBank JX867688), 20080846 (GenBank JX867691), 20080855 (GenBank JX867684), 20081753 (GenBank JX867699), 20083018 (GenBank JX867686). **Tibet**, Nyingchi, Lulangzhen, Dongjiuxiang, alt. 2550 m, on bark, 17 Jul 2011, Y.L. Cheng 20118005C; Lulangzhen, Observation station, alt. 3400 m, on bark, 12 Jul 2011, Y.L. Cheng 20118228. **Yunnan**, Lijiang, Jiuhexiang, alt. 2700 m, on bark, 30 Oct 2008, Z.S. Sun 20081681; Shangri-La, Tianshengqiao, alt. 3500 m, on bark, 3 Nov 2008, H.Y. Wang 20082176, 20082177, 20083800, 20086000, Y.D. Du 20082092 (GenBank JX867702), Z.J. Ren 20082225 (GenBank JX867685), Z.S. Sun 20083227 (GenBank JX867692).

Comments — See *N. helveticum*.

Nephroma subhelveticum H.Y. Wang, **sp. nov.** Figs 5, 6

MycoBank MB 801765

Differs from *Nephroma helveticum* by its crisp thin thallus, its finer marginal lobules, its smooth ridged non-tomentose dorsal apothecial surface, and its lack of lichen substance N1.

Type — China. Jiangxi, Ji-An, Qianmocun, Mt. Nanfengmian, alt. 1300 m, 1 Nov 2010, on bark, H. Y. Wang 20106583 (**Holotype**, SDNU; GenBank, JX867680).

Etymology — Referring to the very close genetic relationship and morphological similarity with *Nephroma helveticum*.

Thallus foliose, very crisp, about 4 cm in diameter. Lobes long and narrow, usually 5 × 10 mm, with fine lobules at margin (0.1 × 0.2 mm). Upper surface gray, smooth; tomentum, isidia, soredia and pruina absent; lobules marginal and laminal, long or round, branched or not. Upper cortex pale, ca. 20 µm thick; photobiont *Nostoc*, ca. 55 µm thick; medulla white, with abundant crystals soluble in water, ca. 55 µm thick; lower cortex pale, ca. 15 µm thick. Lower surface pale or pale brown, tomentose, paler and smooth at the margins; rhizines stubby, sparse. Apothecia common, immersed on lower surface at tips of lobes, kidney-shaped; disc dark brown, 1–4 mm in diameter; dorsal surface with obvious ridges and lobules, without tomentum; margins usually with

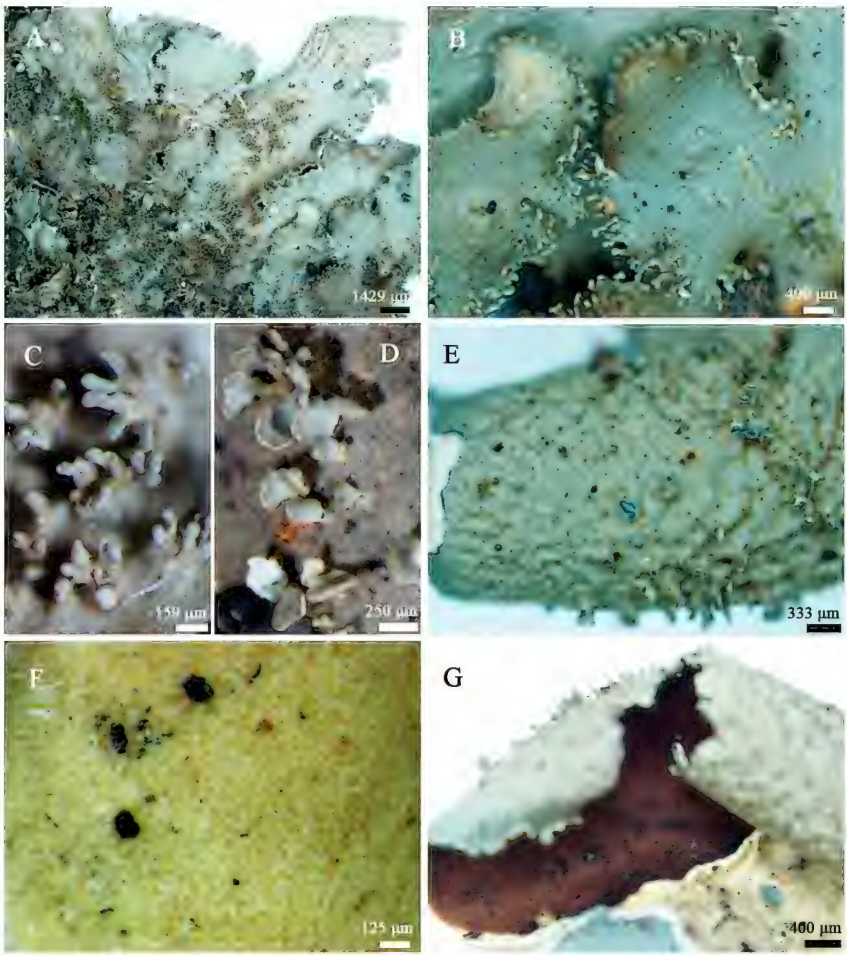

FIGURE 5. *Nephroma subhelveticum* (Holotype, 20106583). A: Thallus; B: Lobe with marginal lobules; C-D: Laminal lobules; E: Smooth and ridged dorsal surface of apothecium; F: Tomentum on lower surface; G: Apothecium immersed on lower surface.

lobules. Epihymenium pale brown, without crystals, ca. 10 µm tall; hymenium hyaline, without crystals, ca. 38 µm tall; hypothecium hyaline, without crystals, ca. 25 µm tall. Paraphyses simple. Asci clavate, I+ blue, 8-spored; ascospores pale brown, 3-septate, 15–20 × 3–4 µm. Pycnidia not seen.

SPOT TESTS—Thallus upper surface: K–, C–, KC–, PD–; medulla: K–, C–, KC–, PD–.

SECONDARY METABOLITES— N2 (FIG. 3)

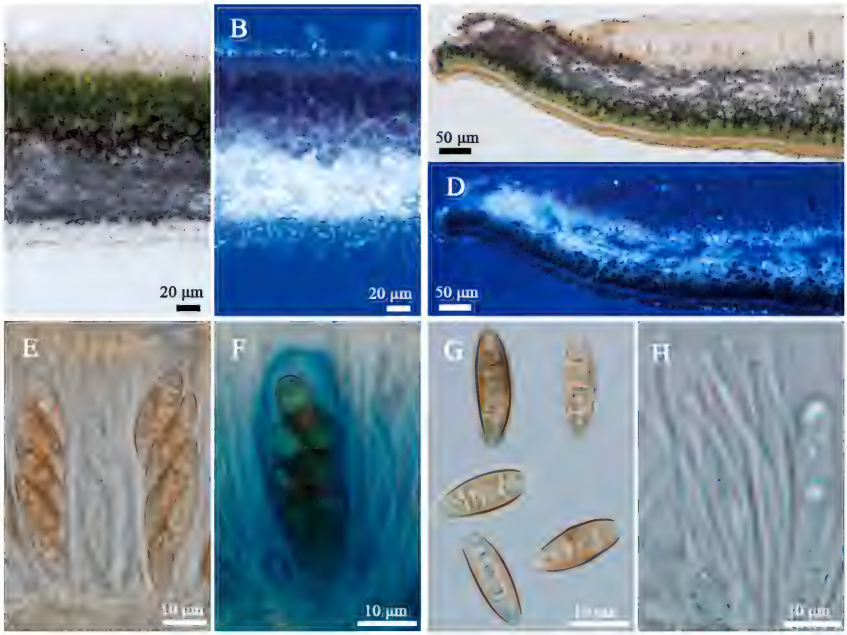

FIGURE 6. *Nephroma subhelveticum* (Holotype, 20106583). A: Section of thallus; B: Crystals in thallus; C: Section of apothecium; D: Crystals in apothecium; E: Asci and ascospores; F: Iodine reaction of ascus and paraphyses; G: Ascospores; H: Paraphyses.

ECOLOGY & DISTRIBUTION IN CHINA — on bark or moss in Jiangxi and Fujian.

ADDITIONAL SPECIMENS EXAMINED — **CHINA**. **JIANGXI**, Ji-An, Qianmocun, Mt. Nanfengmian, alt. 1300 m, on bark, 1 Nov 2010, H.Y. Wang 20106581, 20106595; 2 Nov 2010, H.Y. Wang 20106494 (GenBank JX867679). **FUJIAN**, Wuyishan, Tongmucun, alt. 1200 m, on moss, 25 Oct 2010, M. Li 20105971, 20108510 (GenBank JX867677), 20108511 (GenBank JX867678).

COMMENTS—The crisp and thin (rather than leathery and thick) thallus, the fine (not stout) marginal lobules, the smooth ridged apothecial dorsal surface of apothecia lacking in tomentum (rather than rough and tomentose), and the absence of N1 lichen substances distinguish the new species from *N. helveticum*.

Phylogeny

FIG. 7

The ITS sequence analysis indicates that *Nephroma flavorhizinatum, N. subhelveticum, N. helveticum,* and *N. isidiosum* are very closely related. *Nephroma flavorhizinatum* is a sister group (92% bootstrap support) of the clade including *N. subhelveticum, N. helveticum,* and *N. isidiosum*, with a 0.066–0.095

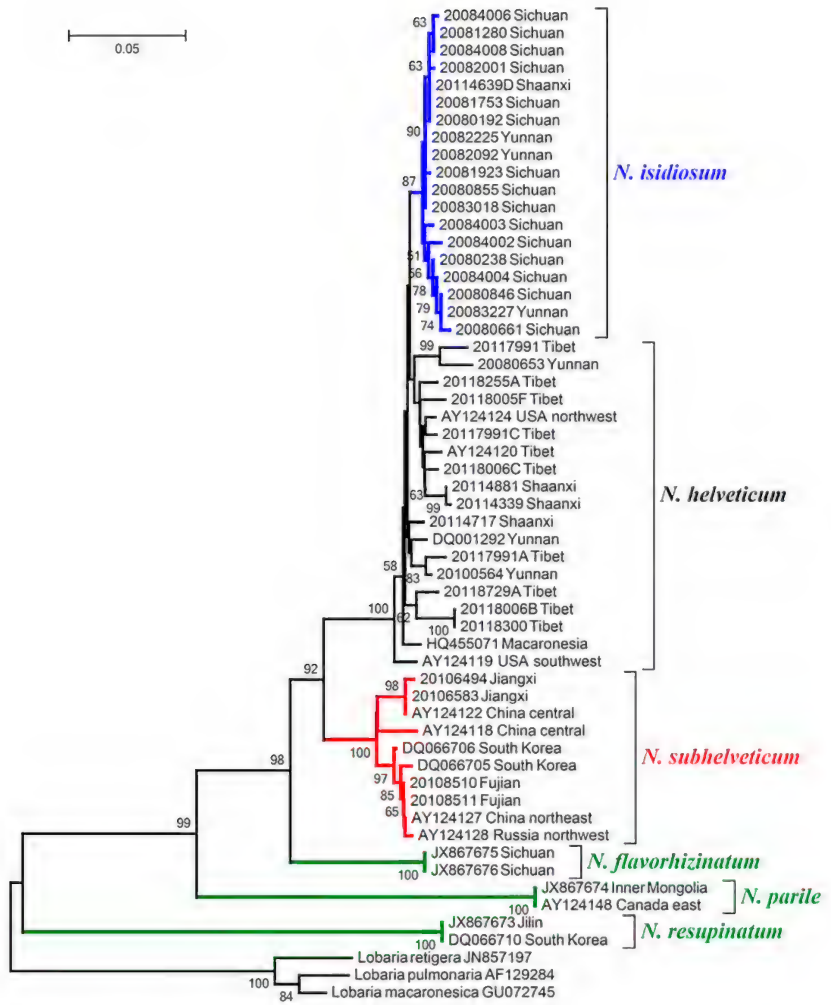

FIGURE 7. The ME tree inferred from ITS data. Bootstrap values >50% (1000 replicates) are shown next to the branches.

evolutionary distance separating sister groups. *Nephroma flavorhizinatum* was first reported from China as a new species differentiated by a golden yellow rhizinal base and distinct pruina at the dorsal surface of apothecia, lobules, and terpenoids (Tian et al. 2011). This morphological differentiation is supported here by the molecular phylogenetic analysis.

Nephroma subhelveticum is sister (100% bootstrap support) to the clade containing *N. helveticum* and *N. isidiosum*, with a 0.046–0.068 evolutionary distance separating sister groups. The *N. subhelveticum* clade (100% bootstrap support) includes the samples from China, South Korea, and northwest Russia; however, the evolutionary distances among these samples are only 0–0.021. The samples within the *N. subhelveticum* sister group are from China, USA, and Macaronesia, among which the evolutionary distances are also very close (0–0.031). These results indicate that the *N. subhelveticum* clade is monophyletic and not conspecific with *N. helveticum*. This result is supported by morphological and chemical characters.

The *N. subhelveticum* clade includes six samples from GenBank (AY1241XX sequences submitted by Lohtander et al. 2002; DQ06670X sequences by Hur et al. 2004). The mtSSU and ITS based research by Lohtander et al. (2002) also included samples of *N. helveticum* and *N. subhelveticum* (identified as '*N. helveticum*') from USA, China, and Russia; their phylogenetic result is identical with our study: two clades, both with >90% bootstrap support. Lohtander et al. (2002) considered that *N. helveticum* might represent closely related taxonomic aggregates. However, they did not compare their specimens morphologically. We have not found detailed information regarding the specimens sequenced by Hur et al. (2004).

The *N. isidiosum* clade is not sister to *N. helveticum*, but rather a child (derived) clade of *N. helveticum;* evolutionary distances between *N. isidiosum* and *N. helveticum* are only 0.009-0.029, suggesting that *N. isidiosum* and *N. helveticum* are conspecific. However, *N. isidiosum* is a monophyletic group with 87% bootstrap support. The genetic diversity level of ITS within *N. isidiosum* is obviously lower than that within *N. helveticum* (Table 2). Moreover, *N. isidiosum* has quite different isidia from *N. helveticum*. These results indicate that all the members of *N. isidiosum* have a common ancestor, and the gene flow between *N. isidiosum* and *N. helveticum* has possibly stopped. In other words, while not conspecific with *N. helveticum*, *N. isidiosum* is a species recently derived from *N. helveticum*.

Table 2. Evolutionary ITS distances within *Nephroma isidiosum* and *N. helveticum*.

Species	Collection locality	No. of Samples	Evolutionary distance (MCL)
N. isidiosum	Shaanxi, Sichuan, Yunnan	19	0–0.011
N. helveticum	Shaanxi	3	0.001–0.015
	Tibet	10	0–0.027
	Yunnan	3	0.009–0.025

Acknowledgements

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Hydnotrya laojunshanensis sp. nov. from China

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Abstract — In August 2012, a solitary *Hydnotrya* specimen was collected under *Abies forrestii* var. *smithii* in the Laojun mountains, Yunnan Province, southwest China. Morphological and molecular analyses showed that this was a new species, which is described here as *H. laojunshanensis*.

Key words — *Pezizomycetes*, taxonomy, ITS, phylogeny

Introduction

Hydnotrya Berk. & Broome (*Pezizomycetes, Ascomycota*) is a genus of hypogeous fungi related to truffles. The genus was placed in *Helvellaceae* by Spooner (1992), but recent molecular analyses support its placement in the *Discinaceae* (O'Donnell et al. 1997; Hansen & Pfister 2006; Tedersoo et al. 2006; Læssøe & Hansen 2007). *Hydnotrya* is distributed across the northern hemisphere including Europe, North America, and Asia (Stielow et al. 2010). There are about 15 species in the genus (Kirk et al. 2008: 325) including a neglected new species, *Hydnotrya bailii* Soehner (Soehner 1959; Stielow et al. 2010). Four *Hydnotrya* species have been reported in China: *H. cerebriformis* Harkn. in Shanxi and Xinjiang Provinces, *H. cubispora* (E.A. Bessey & B.E. Thomps.) Gilkey in Sejila mountains, Linzhi, Tibet, and *H. michaelis* (E. Fisch.) Trappe and *H. tulasnei* (Berk.) Berk. & Broome in Jilin Province (Tao & Liu 1989; Xu 2000). In August 2012 one *Hydnotrya* specimen was collected in the Laojun mountains, Yunnan Province, southwest China (Plate 1). Morphological examination and DNA sequence analyses clearly distinguished the specimen from all other *Hydnotrya* taxa. We describe it as a new species and discuss its relationships with other *Hydnotrya* species.

Plate 1. Location of Laojun mountains, Yunnan Province, southwest China.

Materials & methods

Microscopic and macroscopic descriptions were made of specimen YAAS L2425 following the methods of Bemmann & Bandini (2011). Sections were made with a razorblade by hand, mounted in 5% KOH solution or water, and then stained with cotton blue or lactophenol solution. The sections were observed under an Olympus BH-2 microscope. The type specimen was deposited in the Herbarium of Yunnan Academy of Agricultural Sciences, Kunming, China (YAAS).

Total genomic DNA was extracted using the Zomanbio Plant Genomic DNA Kit. The internal transcribed spacer (ITS) rDNA region was amplified with PCR primers ITS4 and ITS5. The PCR reactions were run on a BIO-RAD C1000TM Thermal Cycles with the following settings: initial denaturation for 5 min at 94°C, followed by 32 cycles of 40 s denaturation at 94°C, annealing at 56°C for 40 s, extension for 1 min at 72°C, and final extension at 72°C for 10 min. Purifying and sequencing of PCR products were conducted by Beijing Genomics Institute.

The *Hydnotrya* YAAS L2425 sequence was analyzed molecularly with 35 retrieved from GenBank were used for phylogenetic analysis. *Gyromitra infula* (Schaeff.) Quél. was selected as the outgroup. Sequences were edited and assembled using SeqMan II (Larsson & Sundberg 2011). Alignment of nucleotide sequences was performed by Mafft-win. Sequences were adjusted manually using BioEdit 7.0.1. Phylogenetic analyses were carried out via PAUP* 4.0 BEAT. Equally weighted parsimony (MP) was used to search for optimal trees, its specific procedures and analyses were following the methods of Hofstetter et al. (2002) and Larsson & Sundberg (2011).

Results & discussion

Phylogenetic analysis

Six strict consensus trees were generated based on the MP analyses of the 36 ITS sequences (L = 607, CI = 0.883, RI = 0.927, RC = 0.818), shown in Plate 2 with associated bootstrap supports for branches.

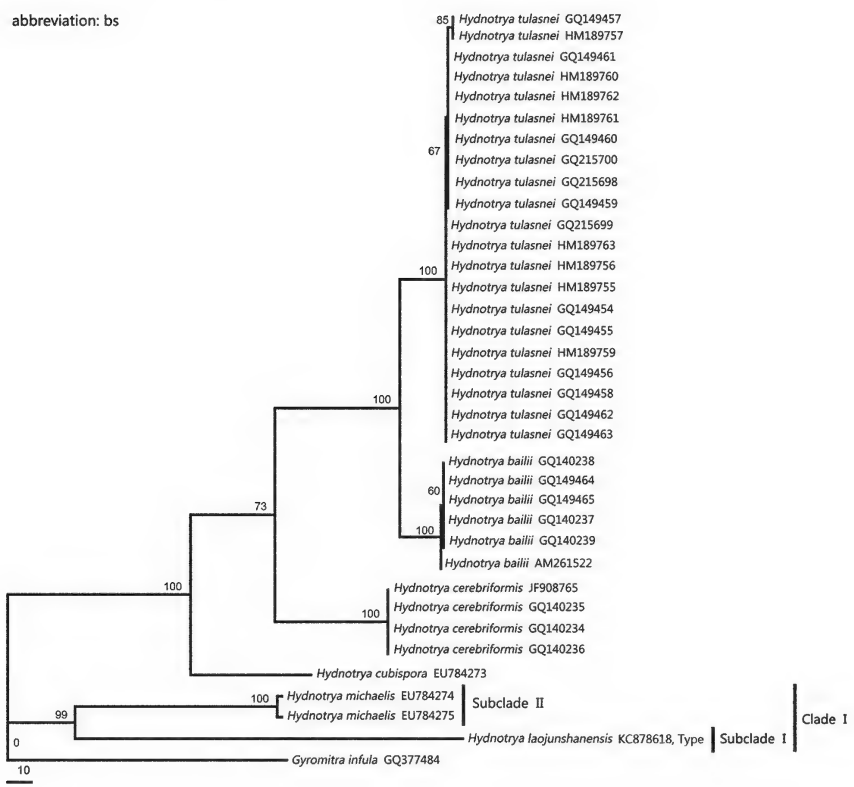

Plate 2. The strict consensus tree produced from ITS sequence analysis of *Hydnotrya* spp.

In the strict consensus tree the new species and *H. michaelis* form Clade I with 99% bootstrap support. This then subdivides into two subclades. Subclade I contains *H. laojunshanensis* and Subclade II *H. michaelis,* which has strong bootstrap support (bs = 100%). The phylogenetic analysis supports *H. laojunshanensis* as separate from (although closely related to) *H. michaelis* and distinct from other *Hydnotrya* species.

Taxonomy

Hydnotrya laojunshanensis Lin Li, D.Q. Zhou & Y.C. Zhao, **sp. nov.** Plates 3, 4

MycoBank MB 803968

Differs from all other *Hydnotrya* spp. by its single-chambered ascocarp and larger ascospores.

Type: China: Yunnan, Laojun mountains, 26°42′N 99°42′E, alt. 3786 m, in forest of *Abies forrestii* var. *smithii* Viguié & Gaussen, 30.8.2012, Lin Li (**Holotype**, YAAS L2425; GenBank KC878618).

Etymology: from the Latin for Laojun, the type locality.

Ascocarp irregularly globose, 1.0–1.2 cm in diameter when fresh, smooth, single-chambered with a primary apical opening up to 0.1–0.5 cm in diameter; cavity wall 1–2 mm thick when dry, of three layers: inner layer white, middle layer red, outer layer reddish-brown. Peridium two-layered, 605–633 μm

Plate 3. *Hydnotrya laojunshanensis* (holotype, YAAS L2425). Ascocarp.

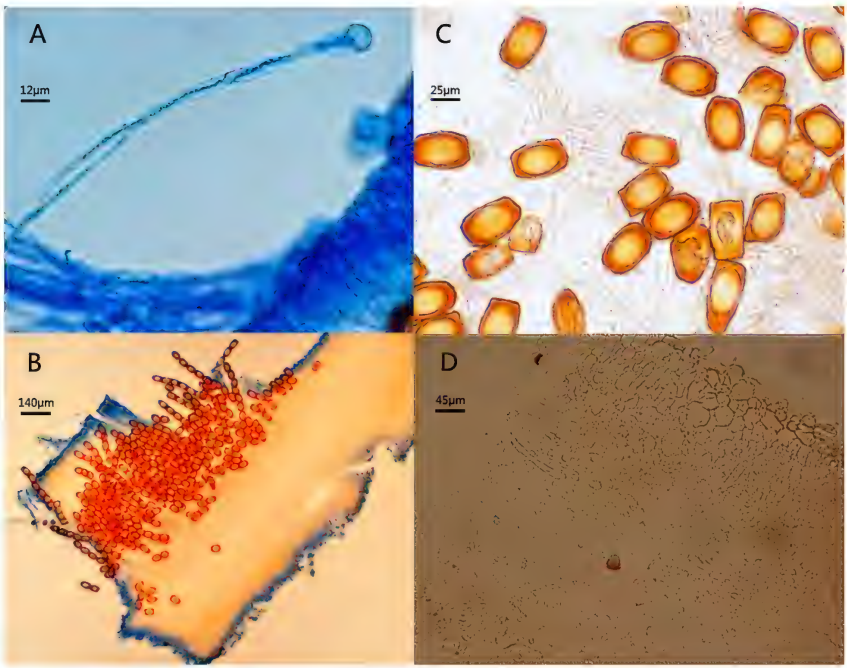

Plate 4. *Hydnotrya laojunshanensis* (holotype, YAAS L2425). A. Paraphyses in lactophenol cotton blue; B. Asci in lactophenol cotton blue; C. Ascospores in water; D. Peridium in lactophenol solution.

thick, outer layer 223–260 μm thick, pseudoparenchymatous, composed of light brown angular or irregular cells of (27.7–)33.0–46.5(–54.0) × (19.7–)31.8 (–45.0) μm, inner layer, 382–373 μm thick, consisting of hyaline interwoven hyphae. Hymenium two-layered, the one next to the peridium deep orange, another yellowish to whitish. Asci cylindrical, 342.4–401.1 × (24.2–)29.1–35.9 μm, 8-spored, regular-monoseriately arranged. Ascospore elliptical (without ornamentation) or rectangle (with ornamentation), reddish orange, (42.5–) 50.0–57.2(–60.3) × (27.5–)30.4–36.9(–38.2) μm (with ornamentation), (29.2–) 35.3–42.4(–44.0) × (20.3–)24.4–29.8(–30.0) μm (with ornamentation), Q = 1.28. Paraphyses hyaline, straight, 2.4–6 μm in diam, apical slightly inflated up to (10.8–)13.1–15.2(–18.3) μm wide, septate, protruding up to 242.6–373.0 μm.

Ecology & Distribution: solitary in the soil in an *Abies forrestii* var. *smithii* forest. Known only from Yunnan Province, China.

Comments — This species is characterized by large, rectangular ascospores and a single-chambered ascocarp, which distinguishes it from all other *Hydnotrya* species. ITS sequence analysis also shows that *H. laojunshanensis* is distinct from

other *Hydnotrya* species, although closely related to *H. michaelis*. *Hydnotrya laojunshanensis* differs in having larger ascospores and lacking any cerebriform folding, whereas *H. michaelis* typically has strongly inwardly folded lobes.

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Phylogenetic divergence of three morphologically similar truffles: *Tuber sphaerosporum*, *T. sinosphaerosporum*, and *T. pseudosphaerosporum* sp. nov.

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Abstract — We describe and illustrate *T. pseudosphaerosporum*, a new species from Yunnan, China. This is most closely related to the European *T. borchii* and the North American *T. gibbosum*. ITS-rDNA sequence analysis reveals the phylogenetic divergence of three morphologically similar truffle species: *T. sphaerosporum* from North America, and *T. sinosphaerosporum* and *T. pseudosphaerosporum* from China. Phylogenetically, *T. sphaerosporum* is most closely related to another North American species, *T. californicum*, while *T. sinosphaerosporum* clusters with a European species, *T. dryophilum*.

Key words — *Ascomycota*, white truffle

Introduction

In November 2012, during our yearly investigation of *Tuber* species in Yunnan Province, China, we found some white truffles in a stand of the conifer *Pinus armandii* Franch. This truffle differed from most known species but was morphologically almost indistinguishable from the North American *T. sphaerosporum* Gilkey (Gilkey 1954) and the Chinese *T. sinosphaerosporum* L. Fan et al. (Fan et al. 2013). Our aim was to resolve the phylogenetic relationships of these three species based on ITS-nrDNA sequences and to describe the new species. Our phylogenetic analysis indicates that the new species is distant from both *T. sphaerosporum* and *T. sinosphaerosporum*.

Materials & methods

Morphological studies

Fresh specimens were collected from Yunnan Province, China, and deposited in the Herbarium of the Biology Department, Capital Normal University (BJTC), Beijing.

Table 1. *Tuber* specimens and sequences used in phylogenetic analysis.

Species	Voucher	Origin	GenBank No. (ITS)
T. aestivum	TaeW064S-W140 (UPS)	Sweden	AJ888062
	TaeW028I-E157 (UPS)	Italy	AJ888118
T. borchii	GB45	Italy	HM485344
T. sphaerospermum	AH39190	Spain	JN392246
T. californicum	JT22590	USA	HM485351
	JT28058	USA	HM485346
T. dryophilum	GB69	Italy	HM485353
	GB37	Italy	HM485354
T. gibbosum	JT2789	USA	FJ809869
T. lijiangense	HKAS 52005 (Holotype)	China	KF805727
	HKAS 52005	China	GQ217541
T. microsphaerosporum	BJTC FAN152 (Holotype)	China	KF805726
T. oligospermum	MA: Fungi: 41010A	Spain	FM205506
	MA: Fungi: 41010B	Spain	FM205507
T. oregonense	JT8767	USA	FJ809879
T. pseudosphaerosporum	**BJTC FAN250 (Holotype)**	**China**	**KF744063**
	BJTC FAN260	**China**	**KF744062**
T. puberulum	TL3857	Denmark	AJ969625
T. sinosphaerosporum	BJTC FAN135 (Holotype)	China	JX092086
	BJTC FAN136	China	JX092087
T. sphaerosporum	JT12487	USA	FJ809853
	JT19772	USA	FJ809854

Macroscopic characters were described from both fresh and rehydrated specimens. Microscopic characters were described from razor-blade sections mounted in 3% KOH, Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. For scanning electron microscopy (SEM), spores were scraped from the dried gleba, placed onto double-sided tape, mounted directly onto an SEM stub, coated with palladium, and then examined and photographed using a HITACHI S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube, together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was then extracted using a EZNA_Fungal DNA kit (Omega Bio-Tek Inc. Norcross, Georgia, United States) following the manufacturer's protocol. The ITS region was amplified by PCR using the primers ITS1 and ITS4 (White et al. 1990). PCR was performed in a 50 µl reaction system containing 2 µl of DNA template, 2 µl of each 10 µM primer, and 25 µl of 2× Master Mix (Tiangen Biotech Beijing Co. Ltd., Beijing). The PCR protocol was as follows: an initial denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 2 min, 55°C for 25 s, 72°C for 2 min, with a final extension at 72°C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data included in our study were downloaded from GenBank (Table 1).

Phylogenetic analyses

DNA sequences were aligned using Clustal X v1.83 (Thompson et al. 1997), and the alignment was manually adjusted using Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed by maximum parsimony (MP) using PAUP* 4.0b10 (Swofford 2002). MP analysis was conducted using heuristic searches with 1,000 random addition sequence replicates, and the tree bisection reconnection (TBR) branch-swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BP) analysis was performed with 1,000 replicates, each with 10 random addition sequence replicates, and using TBR branch-swapping. A Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist & Huelsenbeck 2003) with two sets of four chains

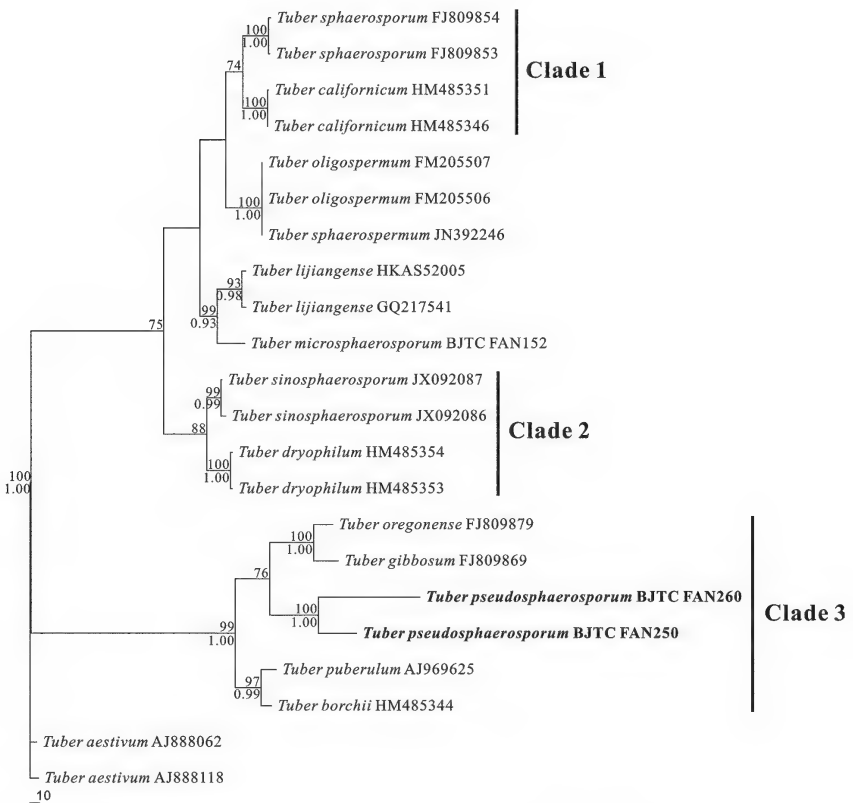

FIG. 1. Phylogeny derived from maximum parsimony analysis of ITS rDNA sequences from some *Tuber* species with globose ascospores. Bootstrap values of >70% are shown above branches. Clades with Bayesian posterior probabilities (PP) estimated at >0.90 are shown under branches. *Tuber aestivum* was used as outgroup. Tree length (TL) = 876 steps, consistency index (CI) = 0.7021, retention index (RI) = 0.7792, homoplasy index (HI) = 0.2979, and rescaled consistency index (RC) = 0.5470.

(one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% of trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. *Tuber aestivum* Vittad. was used as the outgroup.

Results

Molecular phylogeny

Maximum parsimony analysis resulted in one most parsimonious tree (Fig. 1).

Our phylogenetic analysis was based on sequences from 13 *Tuber* species with globose ascospores. It revealed that *T. sphaerosporum*, *T. sinosphaerosporum*, and *T. pseudosphaerosporum* are in separate clades. *Tuber sphaerosporum* clustered with *T. californicum* Harkn. in Clade 1 with moderate support, and *T. sinosphaerosporum* clustered with *T. dryophilum* Tul. & C. Tul. in a well-supported clade (Clade 2 in Fig. 1). A third clade was also strongly supported, and contained *T. pseudosphaerosporum*, *T. oregonense* Trappe et al., *T. gibbosum* Harkn., *T. borchii* Vittad., and *T. puberulum* Berk. & Broome.

Taxonomy

Tuber pseudosphaerosporum L. Fan, **sp. nov.** Fig. 2

MycoBank MB 803932

Differs from other *Tuber* species by its purple-brown gleba and regular globose ascospores with coarse reticulum.

Type: China. Yunnan Province, Huize County, Daibu Town, in the soil under *Pinus armandii*, 11 Nov. 2012, Shuang Feng 017 (**Holotype**, BJTC FAN250).

Etymology: *pseudosphaerosporum* (Lat.), referring to the great similarity with the North American *T. sphaerosporum*.

Ascomata 2–4.5 cm in diam., white, whitish, or whitish-yellow when fresh, subglobose to irregular, mostly much lobed with deep furrows, firm, nearly glabrous. Odor pungent at maturity. Peridium 250–350 μm in thickness, outer layer pseudoparenchymatic, 150–250 μm, composed of subglobose, subangular or irregular cells of 5–25(–40) μm, smaller and larger cells intermixed, cells hyaline with somewhat thickened yellow walls and up to 2.5 μm; toward inner side grading into textura intricata, composed of interwoven hyphae, hyaline, septate, thin-walled, branched, 3–5 μm in diam., occasionally with a few hyphae swollen to 20 μm in diam. Gleba white at first, gradually becoming brown, and finally somewhat dark grey-brown with a purple tint at maturity, marbled with larger whitish meandering veins. Asci 1–3(–4)-spored, hyaline, thin-walled, subglobose to ellipsoid, sessile, 55–85 × 52.5–75 μm. Ascospores brown, regularly globose, reticulate, 25–37.5 μm in diam. excluding ornamentations,

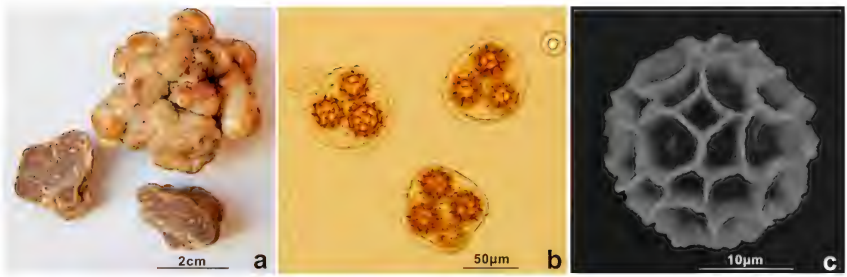

FIG. 2. *Tuber pseudosphaerosporum* (BJTC FAN250, holotype). a. Ascomata; b. Ascospores observed under the light microscope; c. Ascospore observed under SEM.

alveoli large and regular, up to 4–7.5 μm tall and 3–4(–5) alveoli across the spore width.

ADDITIONAL SPECIMEN EXAMINED: **CHINA. YUNNAN PROVINCE**, Huize County, in the soil under *Pinus armandii*, 11 Nov. 2012, Xiao-yong Li 008 (BJTC FAN 260).

COMMENTS—*Tuber pseudosphaerosporum* is morphologically similar to both *T. sinosphaerosporum* and *T. sphaerosporum*. These three species are very easily confused in most of their taxonomic characters (TABLE 2). Our phylogeny, however, places them in separate clades (FIG. 1), indicating their independent origins. The grey-purple tint in the gleba appears to be unique to the new species and is not observed in *T. sinosphaerosporum* or *T. sphaerosporum* (Gilkey 1954; Fan et al. 2013). In addition, the ascomatal surfaces of both *T. sphaerosporum* and *T. pseudosphaerosporum* are glabrous, while *T. sinosphaerosporum* produces poorly developed and non-specialized septate hyphae-like obtuse hairs on its ascomatal peridium (Fan et al. 2013).

Discussion

Tuber pseudosphaerosporum, *T. sinosphaerosporum*, and *T. sphaerosporum* are very similar morphologically, especially in their ascospore ornamentation — traditionally one of the most important characters used for species differentiation in this genus (TABLE 2). All three have large-meshed reticulate ascospores (generally 3–4 meshes across the spore width), dark gleba, and pale colored ascomata with nearly glabrous surfaces. Hypha-like hairs can occasionally be found on the peridia of *T. sinosphaerosporum*, but these are usually poorly developed and easily overlooked. It is thus very difficult to separate these species on the basis of morphology. Our phylogenetic analysis however, clearly places the three species in different clades (FIG. 1), indicating that they have evolved in different lineages despite their morphological similarities.

Table 2. Morphological comparison of the three *Tuber* species.

Morphological features	*T. pseudosphaerosporum*	*T. sinosphaerosporum*	*T. sphaerosporum*
Ascocarp color	Whitish	White to whitish	Pale brown
Ascocarp size	2–4.5 cm	1.5–5.5 cm	2.5 cm
Peridium surface	Glabrous	Nearly glabrous	Glabrous
Peridium structure	Irregularly pseudoparenchymatic	Pseudoparenchymatic	Irregularly pseudoparenchymatic
Peridium thickness	150–250 μm	250–300 μm	—
Peridium hairs	Absent	Cylindrical and obtuse	Absent
Gleba	Deep brown with purple tint	Dark brown	Dark brown
Spore number in ascus	1–3(–4)	1–4	—
Spore shape	Regularly globose	Regularly globose	Regularly globose
Spore size	25–37.5 μm	20–45 μm	32–58 μm, including alveoli
Spore ornament	Alveolate	Alveolate	Alveolate
Alveoli number	3–4(–5)	2–4	3–4
Alveoli height	4–7.5 μm	5–7.5 μm	—

Tuber sphaerosporum is known only from North America, and *T. pseudosphaerosporum* and *T. sinosphaerosporum* are known only from southwestern China.

Acknowledgments

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Spatial data for fungal specimens: retrospective georeferencing and practical recommendations for mycologists

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Abstract — The number of studies based on herbarium data for analyzing biogeographical patterns and environmental questions is increasing, as herbaria are making their collections available online. However, the quality of a specimen's spatial data still varies dramatically among records. Most historical specimen records either lack geographic information or have only vague textual descriptions about the locality, while contemporary records may exhibit unwarranted variation in spatial data quality, requiring increased awareness among mycologists about the importance of high quality primary spatial data for specimens. Georeferencing is the process of assigning geographic coordinates to a record linking it to a geographic location on Earth, and it can be processed retrospectively for records without geographical coordinates based on locality descriptions or directly collected in the field using GPS handheld units. Here we provide an overview of methods for georeferencing historical data retrospectively, discuss practical recommendations for collecting high quality spatial data for fungal specimens, and suggest decimal degrees as a standard form for citing geographic coordinates.

Keywords — biogeography, conservation, ecological niche modelling, GIS, phylogeography

Introduction

Fungal taxonomy aims to investigate the diversity of fungi on Earth, assigning names, and proposing phylogenetic relationships among species. Global estimates for fungi vary dramatically depending on the methods used (Blackwell 2011, Scheffers et al. 2012), ranging from 611,000 (Mora et al. 2011) up to 9.9 million species (Cannon 1997). Presently, there are almost

100,000 known fungal species (Kirk et al. 2008), which surely represent only a small fraction of extant species. Specimens harbored in herbaria represent occurrence records of a taxon at a given location on a specific date and provide a fundamental reference to morphological and molecular studies that are necessary to ascribe species names (e.g., Brock et al. 2009, Osmundson et al. 2013). They allow the delimitation and revaluation of a species identity when more taxonomic and molecular knowledge becomes available (e.g., Cabral et al. 2012). Herbaria throughout the world are increasingly making their collections available online, and consequently the number of studies based on herbarium data for analyzing biogeographical patterns and environmental questions is also increasing (Lavoie 2013). However, most biological specimen records either lack geographic coordinates or have only imprecise textual descriptions about the locality (Guralnick et al. 2006), severely limiting the comprehension of species distributions. Public databases containing fungal specimen data exist at global (e.g., Global Biodiversity Information Facility – GBIF, www.gbif.org/) and regional scales (e.g., *species*Link network, www.splink.org.br/), but the use of this data is still incipient among mycologists.

In the case of fungi, herbarium data have been explored to investigate biogeographical patterns (Wu & Mueller 1997, Mueller et al. 2001, Oda et al. 2004, Wollan et al. 2008, Geml et al. 2012, Wolfe et al. 2012) and climate change effects on sporocarp phenology (Kauserud et al. 2008, 2010, 2012), and to model the potential distribution of invaders (Wolfe et al. 2010, Wolfe & Pringle 2012). However, as we improve our knowledge about fungi, the quality of spatial data still varies dramatically among records. As expected, most historical records have vague textual descriptions of localities, and most labels lack geographical coordinates. Nevertheless, such unwarranted variation in spatial data quality can also be found in contemporary records, requiring an increased awareness by mycologists.

Halme et al. (2012) discussed the need to rethink data collection, database structure, and organization, stressing the importance for standardizing data collection practices. However, they did not emphasize the importance of spatial data quality for documenting species occurrence. The scarcity and variability of data related to geographic coordinates has severe implications for biodiversity and related life science research, including taxonomy, phylogenetics, ecology, biogeography, biological monitoring (Halme et al. 2012), and conservation planning (Dahlberg & Mueller 2011, Molina et al. 2011). Without specimen occurrence information, one cannot make inferences about spatial processes that influence the delimitation and distribution of species. Geographic Information Systems (GIS), which were designed to manage and analyze spatial information, can be used to integrate fungal databases with other variables such

as land cover, protected areas, and climatic layers of present, past, and future scenarios. The development of ecological niche models (Peterson et al. 2011), which use known occurrence points to estimate potential species distribution based on the ecological niche to define suitable sites, is another promising avenue for mycological research. These models are an important biological tool for taxonomy, ecology, evolution, conservation, epidemiology, invasive species management, and protected area planning, as all research fields depend on accurate and precise coordinates for specimens.

Georeferencing is the process of assigning geographic coordinates to a record, linking it to a geographic location on Earth (Chapman & Wieczorek 2006). Legacy specimens, which usually have only textual information regarding their locality, and recent collections made without geographic coordinates may be georeferenced retrospectively (Murphey et al. 2004), increasing the quality of specimen occurrence records. On the other hand, contemporary collectors could improve the accuracy and precision of their geographic coordinates by using Global Positioning System (GPS) handheld units.

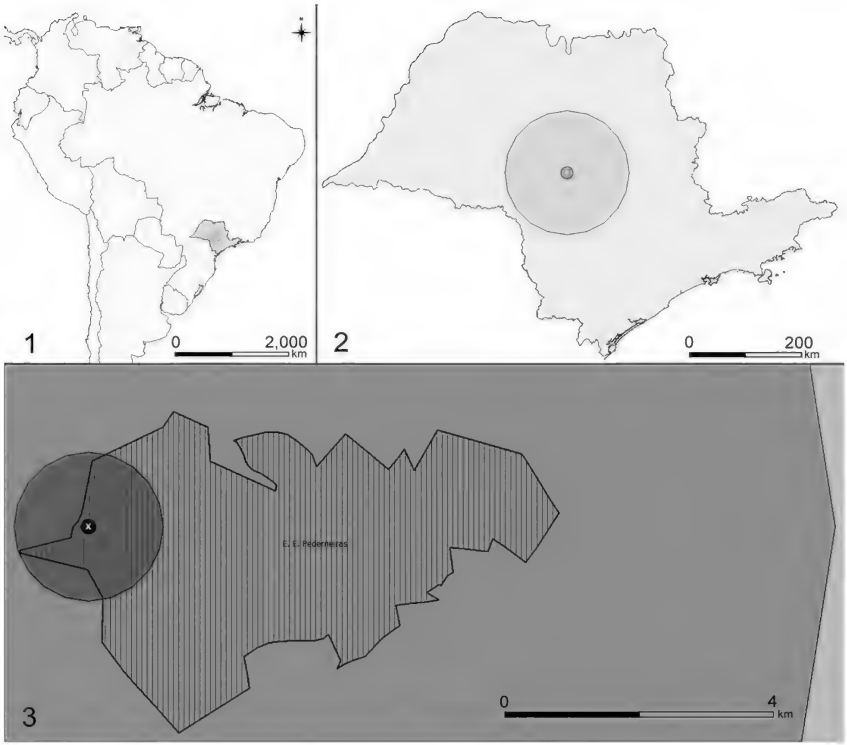
As mycologists may not be well trained in georeferencing, we shall discuss how to improve georeferencing quality for fungal specimens. Our initial motivation came from practical experience, as most fungal specimens now available from the *species*Link network (http://splink.cria.org.br/) — a Brazilian initiative that integrates primary biodiversity data for the Brazilian Virtual Herbarium of Plants and Fungi (http://inct.florabrasil.net/) — lack geographical coordinates. For instance, *Schizophyllum commune* Fr., a widely distributed and quite abundant species, has 410 records in *species*Link network, but only 27 (~ 6.5%) could be selected to generate an ecological niche model. Record selection in this case took into account a number of quality criteria (Giovanni et al. 2012), but it is important to note that most records (85%!) were discarded because of the absolute lack of geographical coordinates. Due to the few records selected and their geographical bias, the generated model omits vast regions in the study area (Braga-Neto 2013a). Similarly, of the 594 records for *Pycnoporus sanguineus* (L.) Murrill available in *species*Link, only 47 (~ 8%) were selected (Braga-Neto 2013b). Therefore, so as to increase awareness about the importance of spatial data for fungal specimens, we provide an overview of methods to georeference historical data retrospectively, discuss practical recommendations for collecting high quality spatial data, and suggest a standard form for citing geographic coordinates.

Accuracy, precision, coordinate system and datum

Understanding the distinction between accuracy and precision is crucial, both for retrospective georeferencing of locality data and documenting

localities (Murphey et al. 2004). ACCURACY refers to how close a measurement of a quantity corresponds to its true value (whereas precision is the degree to which repeated measurements show the same results). Accuracy depends on how data is collected and processed. At the time of collection, accuracy refers to the quality of the location originally reported by the specimen collector, which may be accurately or inaccurately described, regardless the level of geographic detail recorded. In georeferencing, accuracy is related to correctly positioning the locality points based on available information and correctly entering the data into a spreadsheet or database. PRECISION, in the context of georeferencing, refers to the geographic extent potentially represented by the locality (FIGS. 1–3). It is a product of the original locality description or measurement and the georeferencing method applied. A georeference can be accurate but not precise, precise but not accurate, neither, or both. A description of a locality containing only information about the county may be accurate, but because a county is a large geographic area, it is relatively imprecise. On the other hand, a locality described retrospectively by latitude and longitude coordinates may be precise because only a small geographic area is involved, but inaccurate if the georeferencer assigned the values erroneously. Furthermore, a georeference can be inaccurate and imprecise if the coordinate measurement in the field contains a systematic error and the datum was specified incorrectly. The georeferencing process was designed to assign accurate and precise locality data to specimen records, but as all measurement involves some kind of error, it is very important to document uncertainties as they can determine the suitability of data for particular analyses (Rocchini et al. 2011).

Also important is the geographic COORDINATE SYSTEM, which enables every location on Earth to be specified unambiguously. The most common global systems are Latitude/Longitude (preferentially expressed in decimal degrees) and the Universal Transverse Mercator (UTM), which uses a metric-based Cartesian grid to locate positions on the Earth's surface. The UTM system is not a single map projection but a series of map projections (known as zones), one for each of sixty 6-degree bands of longitude. On the other hand, the earth's surface is not perfectly round, so it is necessary to correct these undulations. The DATUM is a mathematical model of the size and shape of the earth, and of the origin and orientation of coordinate systems. Coordinates without a horizontal datum do not uniquely specify a location, so failure to record the correct datum associated with coordinates can result in positional errors of hundreds to thousands of meters on a global scale (Wieczorek et al. 2004). Given its impact on precision, it is essential to provide information about the datum used as an essential part of the coordinate description. Datum shifts must be taken into



FIGURES 1–3. Geographical projections of estimated errors as a function of the number of decimal places in the decimal degree format (Wieczorek et al. 2004). The most precise coordinate was used as a reference point around which errors were depicted as buffer zones; the darker the buffer the more precise. A specimen recorded originally with 5 decimal places (e.g., Latitude –22.33214, Longitude –48.87189) has very precise spatial data, because it includes an error radius of only 1.51 m estimated for this latitude. **1**. If the coordinate chosen as an example did not contain any decimal place (e.g., Latitude –22, Longitude –49) the error dramatically would increase 100,000-fold. This error is depicted as the light gray 151 km radius buffer within São Paulo State, Brazil. **2**. Close-up of São Paulo State showing the same buffer and a smaller and darker one that represents the 15.1 km expected error if the coordinate contained only one decimal place (e.g., Latitude –22.3, Longitude –48.9). **3**. A closer view shows that a coordinate with two decimal places (e.g., Latitude –22.33, Longitude –48.87) is expected to embody an error of 1.51 km, which decreases to 150 m if the coordinate included three decimal places (e.g., Latitude –22.332, Longitude –48.872). The dashed polygon in FIG. 3 represents a Protected Area where the original point was obtained.

account when comparing data of different datum or re-projecting data to avoid the inclusion of errors. The most common global horizontal datum is WGS84 (World Geodetic Survey 1984), but there are some regional datums frequently used, such as NAD83 in North America, and SAD69 in South America.

Retrospective georeferencing of specimen records

Most herbaria that house fungal specimens collected over the past centuries currently face the task of georeferencing a vast amount of historical records, inevitably complicated by imprecise text descriptions, inconsistent formatting, misspellings, old names that have changed, different languages, and even contradictory information about collection sites. Traditionally, herbarium specimen labels have included several levels of text information specifying the site where the collection was made (such as country, county, city, and/or a reference to a place or geographic feature using some measure of distance and direction) but rarely including geographic coordinates. Essentially, retrospective georeferencing is a hypothesis that interprets quantitatively a locality description based on best available geographic information, along with associated uncertainty (Wieczorek et al. 2004). It attempts to define a standardized process by minimizing subjectivity, but it is a time consuming process (Murphey et al. 2004, Guralnick & Hill 2009, Hill et al. 2009), especially if carried out on a specimen-by-specimen basis (Guralnick et al. 2006).

There are some methods being developed to georeference locality descriptions objectively, some of which can be widely implemented even if GIS expertise is lacking (Chapman & Wieczorek 2006). Basically, the methods classify the locality descriptions, determine coordinates and extents, calculate uncertainties, and document the georeferencing process. The Wieczorek et al. (2004) point-radius method provides a relatively easy, practical solution for georeferencing localities and estimating uncertainties. It takes into account aspects of the precision and specificity of the site description, as well as the map scale, datum, precision, and accuracy of the sources used to determine coordinates. Each locality is described as a circle, with a point marking the position most closely described by the site description and a radius describing the maximum distance from that point within which the site is expected to occur. However, the Wieczorek et al. (2004) method tends to overestimate the uncertainty, since it is essentially additive and does not consider the probability distribution for each uncertainty source (Guo et al. 2008); some other methods have developed more complex estimates of uncertainties as probability surfaces (Guo et al. 2008, Liu et al. 2009).

Along with setting appropriate methods, there is a general concern for developing processing tools to increase the rate for georeferencing locations by focusing on automated methods and batch processing (Guralnick et al. 2006, Hill et al. 2009). These initiatives apply documented data standards and provide essential information about the data processing steps to ensure the process is replicable and may be improved upon, as the methods are still being developed (Hill et al. 2009). Even so, Wieczorek et al. (2004) recommend checking

automated results to ensure that they were interpreted correctly. Development of these integrative tools will reduce redundancy of effort by returning assigned coordinates directly to the data curators, increasing efficiency along the process (Guralnick et al. 2006, Hill et al. 2009).

Depending on the data volume to be processed and the world region, another promising and increasingly adopted approach to retrospective georeferencing is based on Google Earth© (Garcia-Milagros & Funk 2010, Năpărus & Kuntner 2012, Weirauch et al. 2012). This convenient and freely available popular software offers a friendly interface that easily allows information visualization, accepts data imported from different sources and formats (including decimal degrees), includes uncertainty and datum, overlays maps, creates paths, points, and polygons, measures linear distances or paths, checks point elevations, manages points and associates them with notes and photos, shares data, and exports to other software. However, the time needed to georeference each specimen may be considerable (Garcia-Milagros & Funk 2010).

Georeferencing specimen records today

Collecting data in the field is the first step towards good georeferencing procedures (Chapman & Wieczorek 2006). The most important improvement in georeferencing practices stems from the Global Positioning System (GPS), a satellite-based navigation system which determines location points anywhere on Earth and in all weather conditions and is freely accessible to anyone with a GPS receiver. Given the high accuracy and precision of GPS devices in recording locality data, we strongly recommend GPS use in the field. All GPS devices provide latitude and longitude, and some also calculate altitude. Since the end of the 'Selective Availability' period on 1 May 2000, the accuracy of hand-held GPS units improved from more than 100 m (McElroy 1998) to less than 1 m.

The most useful devices for biological surveys are hand-held GPS units, which are relatively cheap, small, and effective. Most modern units have a colorful screen that displays map features and allows fast handling, along with 12 parallel channels for acquiring GPS satellite signals faster and more accurately. It is best to choose waterproof or at least water-resistant models with replaceable batteries for collecting specimens in the field. Currently, GPS units do not have basemaps by default, but it is possible to transfer maps and increase their usefulness. For instance, the BirdsEye Satellite Imagery service offers high-resolution color satellite imagery of surrounding environments that at an additional cost can be uploaded to Garmin handheld GPS units. The highest resolution images can help mycologists navigate, find trails, avoid edges in forest fragments, and estimate spatial coverage, among several potential

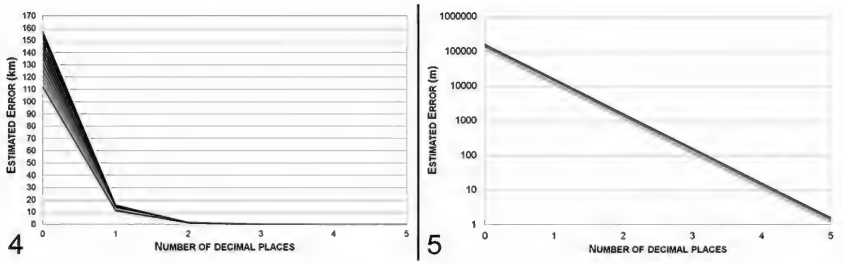
applications. Halme et al. (2012) demonstrated how to estimate sampling effort by recording tracks produced by GPS during an inventory of wood-inhabiting fungi in Finland. All these data require memory space, so devices with a USB connection are highly recommended.

However, GPS data should not replace text descriptions of localities, which are essential for validating the points. In contrast to legacy records, modern collectors have the advantage of being directly responsible for specimen data quality and can use many techniques and tools to ensure accurately georeferenced locations. Hence, site descriptions should be as specific, unambiguous, complete, and accurate as possible (Chapman & Wieczorek 2006). Collectors should avoid using vague terms such as 'near', 'along' and 'center of' without providing an offset distance estimate. To reduce uncertainty, localities to be used as reference points should be permanent sites covering small areas. If the site can only be tracked down by distances measured along a path, road, or river, it is important to be accurate.

If no GPS device was available in the field, coordinates should be assigned as soon after collection as possible from online maps (e.g., Google Earth) or gazetteers (e.g., Alexandria Digital Library, Fuzzy Gazetteer). Some tools are available globally (e.g., Geonames; www.geonames.org/), while others focus on particular regions (e.g., GeoLoc, a web service for finding localities in Brazil; http://splink.cria.org.br/geoloc). Various online maps carry more or less detailed text information, such as forest district names that are often cited by collectors. Such maps are available at the national level (e.g., France: www.geoportail.gouv.fr/accueil; Australia: www.aus-emaps.com/topo.php) or sub-national level (e.g., Bavaria, Germany: http://geoportal.bayern.de/bayernatlas/). Other useful tools are GEOLocate (www.museum.tulane.edu/geolocate/), a platform dedicated to georeferencing natural history collections data, and MaNIS Georeferencing Calculator [(http://manisnet.org/gc.html], mostly helpful in calculating offset distances and errors. It is important to assign the coordinates while the information is still fresh, so you can easily and accurately locate the geographical references.

How to improve accuracy and precision of GPS data

GPS accuracy depends mostly on the type of GPS unit used, the number of satellites visible to your receiver, the strength of satellite signals, the geometric positioning of the satellites in the sky, and atmospheric conditions. There is always a degree of uncertainty that should be associated with any coordinate. A GPS unit uses four or more satellites to calculate your latitude, longitude, and altitude on Earth. However, handheld GPS altitude measurements are usually inaccurate, so the most reliable measurements are horizontal. Maximizing



FIGURES 4–5. The precision of geographic coordinates depends on the number of decimal places in the decimal degrees format. **4**. A geographic coordinate expressed in decimal degrees lacking decimal places is expected to embody an error of hundreds of kilometers. However, errors vary with Latitude; regions at the Equator are expected to include up to 35% more error than the ones near the Poles. **5**. The magnitude of the error, expressed in meters in a semi log scale, is negatively related to the number of decimal places. The error is expected to decrease 10-fold for every increased decimal place, so we recommend recording the most complete available information. The inclusion of five decimal places in all measurements ensures enough precision for most applications.

signal strength and the number of satellites available when measuring will produce the best horizontal accuracies. Ensure that your device reads the signal of at least four satellites, but if possible wait for more satellites to be detected. Most GPS units express signal strength and horizontal accuracy, so wait a few minutes or move to an area with better signal reception, taking note of the distance and direction from the original point. GPS satellite signal may be impaired by solid objects, such as mountains and dense forest canopy, causing errors and even no reading at all. If you are working in a closed canopy area, it is useful to turn on the GPS while still in an open area.

It is essential to configure the GPS coordinate system and datum in advance. We recommend Latitude/Longitude in decimal degrees as the coordinate system, and the datum WGS84 as a standard, as it is geocentric and globally consistent. Since this configuration is followed by most systems, it will also facilitate data exchange. A measurement in decimal degrees to five decimal places is recommended (FIGS. 4–5). Most GPS devices do not directly record accuracy with the waypoint data but provide it in the interface showing current satellite conditions. As the importance of estimating uncertainties also applies to GPS-derived data, we recommend noting the accuracy of each point. To forestall problems, it is a good practice to record GPS coordinates and associated data (decimal latitude, decimal longitude, and accuracy) in a notebook. Most importantly, document your data with proper metadata. Metadata is data about data, describing the data with information about who, what, when, where, why, and how; including the model of the device used is important.

Standard form for geographical coordinates

One of the most critical georeferencing steps (and a major source of error) is data entry. Reduce errors by adopting good practices. The most convenient format for storing and managing geographic coordinates is DECIMAL DEGREES (Wieczorek et al. 2004), which relies on just two attributes (decimal latitude, decimal longitude) and does not include extra symbols, minimizing the chances of transcription errors. Using different variations of the unit symbols in the classical degrees, minutes, and seconds input format may produce errors of more than 1 km (Hans Otto Baral, pers. comm.). Decimal latitude is expressed by positive values to the North and negative values to the South of the Equator, varying from 0 to 90 to the North Pole, and 0 to −90 to the South Pole. Decimal longitude is expressed by positive values to the East and negative values to the West of the Greenwich Meridian, varying from 0 to 180 in the East and from 0 to −180 in the West. If possible, record the coordinates in decimal degrees to five decimal places, as insufficiently precise coordinates can result in uncertainties (FIGS. 1–3). Error is expected to increase 10-fold every decimal place lost (FIG. 5), so we recommend to record the most complete available information. It is essential to provide information about the datum used (e.g., Latitude: −26.92856, Longitude: −49.04896, Datum: WGS84). There are online tools that can help convert different formats to decimal degrees, as Converter (http://splink.cria.org.br/conversor), an open access web-based service developed by the Reference Center on Environmental Information (CRIA) that converts different types of geographic coordinates and datums commonly used in Brazil, and GeoCalc (www.geocomp.com.au/geocalc/), a free software that converts coordinate data files between commonly-used mapping systems in the world.

Final comments

Providing high quality geographic coordinates is an essential step in creating species distribution maps, either by simply plotting the occurrence points on the map or by modelling and projecting the ecological niche to depict its potential distribution (Peterson et al. 2011). The adoption of simple practices guarantees the collection of high quality primary spatial data for fungal specimens. Data quality could be increased even further if herbarium curators and editors of taxonomic, ecological, and conservation journals emphasized the importance of providing geographic data for all specimens. Currently, most fungal taxonomic journals do not require geographic coordinates as essential information of specimens examined, leaving the responsibility of providing locality data to collectors and herbarium curators. We encourage researchers to obtain high quality data in the field and editors and curators to encourage

geographic coordinates for specimens, greatly improving data availability and maximizing their usefulness.

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MYCOTAXON

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Volume 125, pp. 303 *July–September 2013*

Regional annotated mycobiotas new to the Mycotaxon website

Abstract — Mycotaxon is pleased to announce two new species distribution lists to our 'web-list' page covering the *Russulaceae* in Brazil (by Sá, Baseia & Wartchow) and poaceous rusts in Pakistan (by Afshan & Khalid). This brings to 107 the open access mycobiotas now available on the Mycotaxon website <www.mycotaxon.com/resources/weblists.html>.

SOUTH AMERICA

Brazil

Mariana C.A. Sá, Iuri G.Baseia & Felipe Wartchow. Checklist of *Russulaceae* from Brazil. 14 p.

Abstract — A checklist of species belonging to the family *Russulaceae* in Brazil is provided. The list, which includes all species recorded in Brazil up to 2013, cites 3 genera and 41 species covering 21 *Lactarius,* 17 *Russula,* and 3 *Lactifluus* species. Brief descriptions, distribution notes, and references are provided.

ASIA

Pakistan

N.S. Afshan & A.N. Khalid. Checklist of the rust fungi on *Poaceae* in Pakistan. 17 p.

Abstract — This paper presents a checklist of rust fungi on family *Poaceae* in Pakistan together with their known host plants. A total of 80 taxa are mentioned here including 65 species of genus *Puccinia* and 15 of *Uromyces*. A host index is also provided.

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bad taxonomy
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